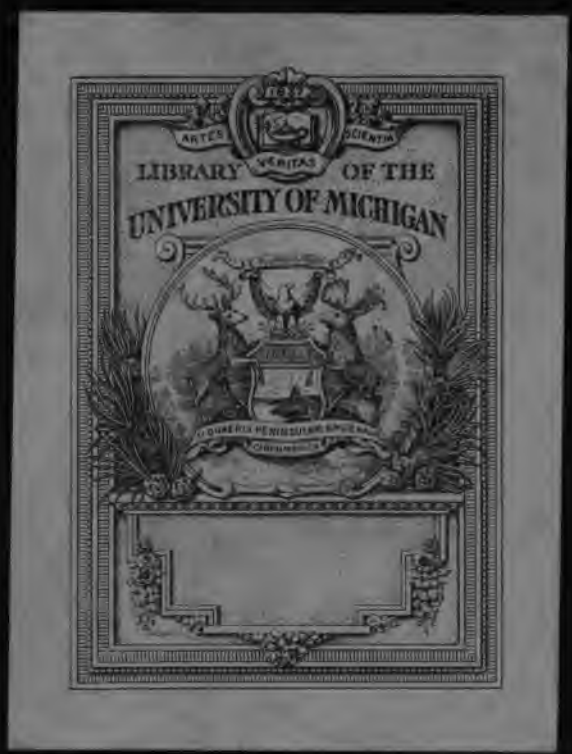
PATHOLOGICAL MYCOLOGY

SECTION I.—METHODS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PATHOLOGICAL MYCOLOGY.

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AN ENQUIRY INTO THE ETIOLOGY OF INFECTIVE DISEASES.

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SECTION 1—METHODS.

WITH SIXTY ILLUSTRATIONS.

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PREFACE.

In the following pages it has been the Authors' endeavour to place clearly and succinctly before their readers the methods employed in the examination and cultivation of Micro-Organisms, especially of those that are supposed to have any relation to disease.

Their object in so doing is twofold: first, to supply to the worker in the field of Scientific Medicine a handy guide, to which reference may be made for instruction and details which can otherwise be obtained only from scattered papers and treatises in various languages; second, to show how extremely simple are most of the methods of research, and thus encourage, as they themselves have been encouraged, young Pathologists to work in a domain, which daily, as it yields new facts to the patient explorer, discloses greater potentialities.

They know how difficult it is for the busy practitioner to devote time to work which, though simple in its details, requires close and continued attention; but they would point out that artificial cultures are easily made, that tissues may be readily preserved for examination, and that those who devote themselves to the subject are usually ready to supply the materials for these cultures, and to conduct the further necessary examination. As the writers have for some years been endeavouring to collect material and statistics in connection with the subject of the relations of Micro-Organisms to

Medical and Surgical Diseases, they now venture to express their willingness to supply materials for making artificial cultures, and to report on material sent to them for examination.

They desire to record their sense of indebtedness to the officials of the German Imperial Board of Health Laboratories, to whose assistance and courteous interest they owe their first acquaintance with many of the facts contained in this section.

They have also to acknowledge the valuable assistance of Mr. J. Tatham Thompson, F.R.M.S., whose exact delineations of microscopic and naked eye appearances have greatly enhanced the value of the descriptive portions of the work; and at the same time they heartily thank Mr. W. B. Mackay, M.B., M.R.C.S., for several valuable drawings.

The drawings not initialed are copied from photographs taken for the purpose.

UNIVERSITY OF EDINBURGH,
June 1885.

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CHAPTER I.

INTRODUCTORY.

General Aspects.

1. Since the theory of spontaneous generation has been abandoned, through the classic researches of Cagniard-Latour and Schwann at the one extreme, and the more recent investigations of Pasteur, Lister, and Koch at the other, the various groups of the organised ferments have become endowed with an interest far greater than any with which they might be invested from their merely biological aspect. They have assumed an almost vital importance from the fact that many of them are now believed to play a prominent part in the causation of certain morbid conditions, both in plants and throughout the whole animal world; and they have come to be recognised as most important factors in such conditions,—factors which must be carefully accounted for in our consideration of many pathological processes, factors which have been studied closely in connection with certain pathological and clinical phenomena, and which not only help to explain these phenomena, but in some cases furnish the only reliable explanation. That these organised ferments—or micro-organisms, as they are termed—do occur in very intimate relation to certain infective diseases has never been doubted, since the means of their recognition have been at our command. A serious discussion has arisen, however, as to whether the presence of these micro-organisms is indicative of a causal or a purely accidental relation between them and the morbid phenomena which appear in their host. In no scientific

problem has the building of hypotheses on other than the basis of exact observation been more discredited, and the principle more firmly established that the only rational method of search for any cause is the close scrutiny of effects, within the confines of which the cause must necessarily be traced. It thus happens that this discussion has been already limited in many directions by the results of careful investigation of all the circumstances which occur in such cases; and it is now held to be indisputable that in certain forms of disease micro-organisms do play the part of the exciting cause. It is of paramount importance to note what have been the means employed in attaining this result, and to inquire whether the same principles applied to the investigation of other diseases in which micro-organisms occur may not lead to a more distinct and accurate conception of their real significance. In so doing it will be found necessary to enter somewhat minutely into details connected with the vital peculiarities of these lowly organised forms, and to describe in order those methods of cultivation and observation which the experience of specialists in this domain of science has pointed out as the simplest and most effectual means of becoming acquainted with their more important characteristics.

True Position of the Subject.

2. Hitherto the work has been carried on by experts only, and the medical profession has been divided into two distinct, but equally illogical, factions. There seems to be a general disposition on the one hand to "pooh-pooh" the very name of micro-organism, whilst on the other there is only too good an example of excessive zeal. The former section scouts the notion of any connection, direct or indirect, relative or absolute, between micro-organisms and disease, of whatever nature. The members of this section have usually had but scant opportunity of studying the evidence now at our disposal, and still less have they tried to settle the matter by a thorough personal investigation. The whole matter is summed up by them as a prevailing "medical fashion," and is cast aside as utterly unworthy of serious consideration. The second section, it is much to be feared, has in great measure to answer for this antagonism. Its

members read the current literature, they quote experiments, excellently planned and carefully carried out by the originator, to prove theories which in the mind of the originator never came to birth. They use the bacillus indiscriminately, they make it the "be all and end all" of disease. If a bacillus or a micrococcus is found, they search no further, they are fully convinced, and all pathological processes may be included in this single word "micro-organism." It is not by such work and such reasoning that the position now attained has been won; no brilliant flash of genius alone has cast the present flood of light upon the relations of germs to disease. Patient observation, carried on by a few ardent and enthusiastic workers, and the gradual and tedious accumulation of facts, combined with careful and sound deductions, alone could place the "germ theory of disease" upon its present solid and logical basis. Solid and logical as this basis is, it must still be amplified in many directions. The work carried on, up to the present by the few, must now be taken up by the whole medical profession, or by as many as are anxious to determine the true position of the question. The amount of material at command is enormous; the methods have gradually been so simplified that the busiest practitioner may help in the work, and by so doing disabuse his mind of many exaggerated notions as to what is actually proved and held by our modern authorities as to the relations between micro-organisms and disease—in the first place generally, and secondly in certain specific diseases.

Relations of Micro-Organisms to the Tissues.

3. It cannot be too strongly insisted upon at the outset that the presence of a micro-organism in the body, or in one of the cavities of the body, may have not the slightest significance from an etiological point of view. We are covered with them, the respiratory and alimentary tracts are loaded with them, and it is not wonderful, therefore, that we should be able to detect their presence on these surfaces. If this fact is borne in mind, and when the numerous means of entrance by way of the free surfaces, cutaneous, intestinal, &c., are considered, one is inclined to marvel that they are not normally present in enormous numbers in the deeper tissues, and that they are present only

under certain abnormal conditions. The invasion of the living body by micro-organisms is a subject which presents great difficulties as to its *rationale*, and as to the exact manner in which it takes place. In regard to the factors which seem to militate against their entry, Lister has pointed out that the chief peculiarity of the tissues, in this direction, is their vitality. In normal conditions the ingress and progress of micro-organisms are checked by the direct vital reaction of the tissue elements themselves. He further insists that not only is this the case as regards healthy surfaces and the deeper tissues, but that two perfectly healthy mucous membranes or fresh cut surfaces, when brought into close apposition, will prevent the development between them, and even the entrance of micro-organisms—an explanation consequent upon the phenomena observed in the urethra, and in connection with healing by first intention. Tissues, then, of what may be termed a Standard Vitality, resist perfectly the entrance of the invading organisms; but let this standard be but to the least extent lowered, and the chance of an invasion at once becomes great. The question is one of intricate physico-chemical relations between the organisms and the tissue elements of their host, which we cannot at present define more exactly than in the preceding general terms. But in looking to the conditions and duration of the invasions themselves, many other points can be established. If it be admitted that this theory of Standard Vitality is correct, it follows, that under normal conditions these minute organisms would be continually excluded from the tissues of the body; but, unfortunately for us, there are acting constantly certain very powerful allies of these micro-organisms, through whose aid the vitality of the tissues is lowered. The general vitality of the body may be depressed by cold, want of food, want of sleep, and irregular habits. Even fear, anger, or other strong emotion may act in the same way by altering both secreting tissue and secretions, thus interfering with the proper nutrition of the tissues, and lowering their vitality.

Means of Ingress.

4. Further, in the altered secretions, micro-organisms normally present may be able to multiply much more rapidly than in the normal secretions, or certain varieties which are not usually present, or are

present only in inconsiderable numbers, may make their appearance in enormous masses, often to the exclusion of those usually found. This is especially the case where, in addition to altered secretion, there is imperfect removal of excreta. Hence even mechanical conditions may have considerable influence in determining the reactions of the tissues and the micro-organisms upon one another, as constrictions, pouches, cavities, and wounds, internal or external, may, any of them, give rise to an accumulation of fluids or excreta which act, firstly, by weakening the tissues, and, secondly, by affording nidus and pabulum to organisms, which, without such a resting-place, could find no base of operations even upon the weakened tissues. The importance of the structural peculiarities of a part in relation to its power of resistance against the attack of micro-organisms, can only be fully appreciated after a careful consideration of these peculiarities. The structure, function, and arrangement of the epithelium covering the surface, the position of the surface, and its relations, first, to the external world, and, second, to the deeper tissues, especially to the lymphatics, the number, size and relations of these lymphatics, the tissues in which they occur, the density, vascularity and vitality of these tissues, and the relations of the lymphatics to the small veins, are, amongst others, points to which special attention must be paid.

If tuberculosis be regarded as a specific infective disease, due to the presence and activity of a certain minute organism, how is it that certain individuals are attacked whilst others escape, that in those attacked the organ most frequently assailed is the lung, and that the selective process passes even further, and that the apex of the lung is specially affected? We can readily understand that the mouth, fauces, trachea, and air passages generally are not affected, because there is a continual emptying of these cavities, a constant ebb and flow of air, and also of the materials which this air carries with it, keeping up a state of unrest—a state which is fatal to the growth of most micro-organisms. This tidal change extends to all parts of the lung where there are proper expansion and contraction, which, as pointed out by the older physicians, fails first at the apex, the position in which phthisis commences. In children, where there has been great enlargement of a bronchial gland, or where, from any other cause, there has been pressure upon a bronchus passing to one of the

lower parts of the lung, the tuberculosis appears in the area in which, owing to the obstruction of the bronchus, there is imperfect entrance and exit of the air, in which, in consequence, there is an accumulation, not only of what enters, but of what should normally escape Should a so-called tubercle bacillus effect an entrance, it has everything in its favour. It is not readily dislodged, it is at rest, it is supplied with ample food stuff, it has an altered epithelial surface on which to act, and it is on a surface between which and the neighbouring lymphatics there is the closest communication. On the other hand, in the respiratory tract, in addition to the above-mentioned conditions, there is in the mouth a thick layer of squamous epithelium which can withstand the attacks of the most vigorous bacillus (unless it develops with extreme rapidity), and so act as a barrier against the entrance of the bacillus into the lymphatics. In the lower parts of the respiratory tubes the epithelial barrier consists of well-formed ciliated columnar cells, the function of which is to pass on from one to another, along with mucus and other material, the bacillus which is seeking lodgment for long enough time to be able to develop morphologically and physiologically, and invasion in this locality is prevented. We shall return to the tubercle bacillus for further illustration and comparison.

Action on Tissues.

5. If it be granted, for the sake of argument, that the germ theory of disease is correct, in what way is it possible to account for the varied changes, local or general, by which an organism manifests its presence?

In certain cases we have an organism whose influence on the system of its host is purely of a local nature, being itself circumscribed in its area of invasion, beyond which neither the organism itself nor its products have any effect. Of such a class the soft sore virus may be taken as a type. On the other hand, there may be strict limitation of the organism to a definite area or tract, beyond which it cannot penetrate, whilst its influence in the form of irritative products may extend throughout the invaded organisation, often with the gravest results. Such a class is represented in all probability by the organisms present in cases of septicæmia. In a third class, the organism is not limited in its sphere of activity, but penetrates

to all parts of the tissues of its host, carrying its irritative properties with it, and producing a general disease which puts to the test the vitality of the affected organisation as a whole. To this class belong the organisms which produce the very varied symptoms met with in the specific fevers. In any of these cases the direct relation of the micro-organism to the tissue elements of its host is probably a dual one. In the first place, the mere fact of its presence as a foreign body amongst the tissues gives it the character of a mechanical irritant. In addition, it may be that one of its vital peculiarities is to evolve an irritating product or *ptomaine*, which, in virtue of its chemical constitution, either irritates the adjacent tissues, in which it may produce a local necrosis, or, as in certain cases, its poisonous effects are spread through the whole of the tissues of the body. It may be that there is, in addition to these two methods of irritation, a more subtle influence exerted by the organism upon the tissue elements of its host, a physico-chemical or molecular reaction occurring between the two, which gives rise to local or to general changes.

Acute Abscess—Tubercle.

6. The marked difference which exists, and which is much greater than at first sight appears, comes into bold relief if the stages in the formation of an acute abscess are observed on the one hand, and those of tubercle formation on the other. In the case of an acute abscess, an indurated mass of tissue is first noticed. If this be punctured, no pus escapes; the tissue reaction has not yet reached the stage of pus formation, but a drop of perfectly clear serum exudes from the centre of the mass. On examination this is found to consist of a few lymph cells and immense hosts of chain or cluster-forming micrococci floating in the lymph. If this puncture be repeated in twenty-four hours pus is found to be present in small quantities, loaded with the same organisms. If repeated punctures be made at intervals, an increased quantity of pus is found each time, and the organisms from the commencement of pus formation begin to decrease, till, in an old abscess of some weeks' standing, no living micro-organisms are present; but the pus is found to contain dead micrococci, not readily recognisable, as they are partially broken down and take on staining reactions very

feebly. The reason of this sequence of phenomena is, that after attaining their maximum degree of development in a locally devitalised portion of tissue, the micrococci are prevented from further progress by the zone of fully vitalised tissue around; and the powerful vital reaction in this zone hems in the organisms on every side, and at the same time limits the formation of pus to the centre of the affected locality. The micrococci soon exhaust all the supplies of pabulum at their command, and then rapidly die out, being in many cases destroyed by their own effete products in the pus; and in pus taken from long unopened abscesses they have quite disappeared. Thus the presence of micrococci in the tissues precedes the reactionary changes to which they give rise locally in the case of acute abscesses. It is an easy enough matter to say that the tissues in which the abscess formed had been devitalised by a blow or by exposure to cold. Either of these, or both, may be cited as the cause, but how do the micro-organisms come to be present in the body? Have the altered conditions of internal surfaces nothing to do with this? Have altered secretions and accumulations of excreta played no part in the introduction of the foreign organism? In fact, under the above conditions, have not numerous organisms from time to time been introduced into the tissues, but so long as the vitality of these tissues was above a certain standard, the "germs" were paralysed or destroyed, and no harm resulted? In those conditions, however, in which abscess formation is met with, these organisms have sought out the devitalised point and have attacked it, and there is a contest between the weakened tissue and the micro-organism. There results an enormous proliferation of cells, many of which become devitalised, and form pus, whilst those at the margin of the abscess of higher vitality prove too strong for the micrococci, and the abscess remains localised. By what means the micro-organism retains its vitality until it reaches the weak point is at present merely a matter of conjecture. Is it simply paralysed, or is it passed rapidly to a spot in which it can live or flourish, or is it so protected by the material in which it had originally flourished, or by other micro-organisms which, themselves dying, still serve as shields to those which arrive at their destination? This, and numerous other most interesting questions, still await solution.

7. Returning to the tubercle bacillus, as an example of an organism acting first chemically on the tissues by its products, and then upon the cells weakened by this reaction, it may be observed that a single tubercle bacillus coming in contact with a healthy mucous surface is unable to retain its footing for a long enough time to allow of the performance of any pathogenic function. In the lung, such a solitary bacillus, even if allowed to remain, could find no food from which to elaborate its own peculiar products, and until this could be done, there would be no changes in the cells with which it is in contact. If, however, a mass of caseous material from some tubercular focus be introduced into an air vesicle or a small bronchus, the conditions are altogether changed. Such a mass, containing the bacillus, its food, and its products, is brought directly into contact with the epithelial surface, and unless the epithelium is endowed with the greatest vitality, it cannot withstand the attacks first of the products of the bacillus, and, second, of the bacillus itself.

That this is not merely hypothetical, is evidenced by the fact that in a case of acute tuberculosis of the lung the following distribution of the bacillus may be observed.

Taking the centre of a lobule, usually the bronchus, as the point from which to commence the observation, it will be noted that in this bronchus, and in the air vesicles immediately surrounding it, caseation has become a well-marked process. In the caseous mass bacilli are found (in the specimens under consideration) in enormous numbers. Passing from the caseous centre, it will be found that where traces of the original structure exist, the bacilli are still present, but are not so numerous as in the centre. Extending the observation to the margin of the tubercular mass, it will be noticed that there is an area of active cell proliferation, not confined to the endothelium of the lymphatics, but appearing also in the epithelium of the air vesicles. In this mass of proliferating tissue there are absolutely no bacilli at first; a few may be distinguished, here and there, near the proliferating area, but where the proliferation is most marked the bacilli are absent. In a very large number of sections examined this was invariably the case. This can be best accounted for on the theory that the bacilli during their growth and development secrete or excrete a chemical substance which alters the

activity or vitality of the cell from its original resistant and stable condition to one in which there is an increased vegetative activity, but a diminished resistant power to the action of the bacillus itself. The highly organised proteid of the cell is thus rendered available as food for these minute organisms, which advance in the track of their chemical vanguard, and continue the process of disorganisation and disintegration.

In this process may be recognised a most beautiful example of the reaction of the bacillus and its products on the tissues, and, in turn, of the tissues on the bacillus.

Tissue Reaction.

8. From recent researches on the mode of reproduction in certain saprophytic and other fungi,[1] it appears that it is possible for a parasitic fungus to derive a sufficient amount of vitality from its host to enable it to reproduce its like without the aid of a sexual process, the necessary stimulus being supplied by the highly organised proteids which are derived from the host, the original imprint of sexual power being again brought into action without further fertilisation. Applying this theory to the tissue cells in which the chemical products appear to set up proliferation, does it not seem more than probable that these cells act the part of female cells, on which a power of reproduction was imprinted at the first impregnation of the ovum, but which is only called into play on the application of a stimulus, probably only chemical (see work of the two Darwins on action of chemical substances on sensitive and insectivorous plants), — a stimulus which thus takes the part of the male element in the reproductive process.[2]

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[1] "On the Sexuality of the Fungi:" H. Marshall Ward, *Quart. Journ. Micros. Sci.*, April 1884.

[2] Professor Frankland has recently defined a plant as being "an organism performing synthetical functions, or one in which these functions are greatly predominant; an animal as an organism pefrorming analytical functions, or one in which these functions greatly predominate." He classes micro-organisms among animals, as "their life essentially depends upon the taking asunder of more or less complex compounds, resolving them into simpler compounds at the expense of potential energy."

It would be almost impossible to classify some of the higher fungi as animals, but

9. As is now well known, cell proliferation may be set up by very numerous and very various forms of irritants, but the results, though varying greatly in degree, are absolutely one in kind. Apply any irritant to a surface or a tissue, and the result is a proliferation of the cells. This appears to be the case especially in the connective tissue group, where the increase in the number of cells is extremely marked. Further, with this increased cell formation there is apparently a taking up of the irritant material by the cells, and if one cell is not sufficient for the task, a number combine to form plasmodia (practically giant cells). That the taking up of these particles or products further lowers the vitality of the cell, is evidenced by the fact that as soon as the cell becomes filled with particles, it dies,[1] and with its contained material acts as an irritant to other cells. The chemical products, then, may be looked upon as the prime movers in bringing about a proliferation of the cells with which they come in contact, by which proliferation the cells appear to attempt to get rid of the irritant material, and their resisting power is thus greatly weakened. At this point the direct action of the bacillus on the cell comes into play. The bacillus attacks the weakened cell, and applies to its own use the rich store of proteids contained within the highly organised but comparatively non-resistant cell. By the aid of this store of proteids it acquires sufficient energy to reproduce its like by asexual spores, the tissue cells are gradually reduced to the peculiar caseous nodules so characteristic of tubercle masses, the degree of caseation in many cases corresponding apparently to the number of the bacilli. The physiological products are sent into the surrounding tissues, and the process is repeated. From this caseous centre the bacilli with their products may be carried to mucous surfaces, and thence to the lymphatics, and even veins; or they may make their way directly to the lymphatics, where in some

some of them certainly exert analytical functions before their synthetical functions come into play. And it can scarcely be held for a moment that the Darwins have proved that insectivorous plants are animal because they flourish so much more luxuriantly when they have presented to them complex proteid substances on which they also certainly exercise their analytical functions before their synthetical functions are called forth.

[1] Metschnikoff, "History of Inflammatory Process:" *Quart. Journ. Micros. Sci.*, January 1884.

cases, as in the small portal spaces around the veins of the liver, they may be found in very considerable numbers, with apparently very little caseation. This caseation, however, advances rapidly.

Digestive Ferments.

10. Duclaux, as a result of his brilliant experiments, arrived at the conclusion that bacteria act upon complex nitrogenous bodies much as do some higher organisms (animals) by a process of true digestion, and that in the process of caseation the organic ferment secretes a material which has much the same action upon milk as the diastase secreted by the pancreas.

Further, Pasteur supposes that no digestion can take place without the presence of micro-organisms, by whose aid alone albuminoid substances are gradually transformed through a series of analytical processes into comparatively simple and readily absorbed material, either in the presence of oxygen, or in some cases where there is no free oxygen present. Only when these soluble substances are formed can there be any food material which the micro organism can apply to its own use in carrying on its nutrition and development. Digestion may therefore be looked upon as a process of fermentation, in which the micro-organisms do not utilise the whole of the material on which they act, but set free a very large proportion, which may be utilised by the organisation in which the process is carried on. The organisms are various, and the products, though in the main similar, contain, as a rule, a minute quantity of a specific substance, and it is this specific substance which, in the pathogenic species, appears not only to digest dead material, but also to prepare living or only slightly devitalised tissues for digestion.

In old tubercle masses the result of this process appears in the form of caseous *débris*, from which all tubercle bacilli may have disappeared, as they have selected what they required, and have exhausted the materials which were necessary for their maintenance; but whilst doing this they have, by setting free their diffusible ferment, prepared the tissues in the immediate neighbourhood for the reception of their progeny. In this way the process is continued and extended. Continuing the analogy between the action of pathogenic

organisms and those concerned in the process of digestion and fermentation, it may be pointed out that after one micro-organism has completed its task another may step in and continue the process of breaking down. How frequently a pyæmic condition supervenes on a tubercular. How often has a patient suffering from tubercular abscess of the kidney or of the lungs, succumbed at last (if not carried off by acute tubercular disease) to pyæmia, and pyæmia in which the symptoms are extremely well defined, where a poison much more active and far reaching in its character than the tubercular poison is rapidly formed on the introduction of a fresh organism into dead, but hitherto inactive or non-irritant, structures.

That these special products of bacterial fermentation do occur can scarcely now be denied, nor that, where the ferment is found, the organism secreting it must first have been present, not necessarily in the position in which the ferment is found—for the ferment may be much more diffusible, and may pass from point to point much more readily than the organism to which it owes its origin, but at some point whence absorption of the ferment could take place.

Specific Differences of Action.

11. The activity of the ferment undoubtedly varies greatly in different cases, and the results of the action of these ferments on the tissue must vary, perhaps not so widely as the ferments themselves, but so far that they may be roughly classified. On the one hand, there are the effects produced in the so-called "granulomata," where an irritant action is set up, followed at first by a proliferation of the connective tissue or other cells, and then by a breaking down and caseation of the proliferated mass, the appearances varying according to the tissue affected and the position of the growth in this tissue. These changes take place slowly. Going to the other extreme, cases of acute septicæmia are sometimes met with, in which the patient is carried off in the course of a few days, and where the *post mortem* changes are of the slightest and most general character. In such extreme conditions there are, on the one side, the slowly growing and slowly multiplying bacilli, which give rise to an irritant product, but one the action of which is fairly definitely localised, and is carried on

for a considerable period, giving rise to marked histological changes before the whole organism is appreciably affected; whilst, on the other side, the ferment is formed rapidly by organisms which grow rapidly, and once formed, this ferment has an extremely toxic action—an action which makes itself manifest at a very early period throughout the whole of the organism attacked.

Between the two extremes are such conditions as pyæmia, where there are not only local manifestations in the tissues after death, but also the acute toxic symptoms during life, in which case, too, the disease runs an intermediate course as regards its rapidity. As pointed out by Koch, these effects depend upon two elements—*first*, the rapidity of growth of the micro-organism which is attacking; *second*, the rate of secretion, and the quantity of the toxic element required to have (*a*) a local and (*b*) a general effect. The rate of growth of the different micro-organisms may be readily observed in artificial cultivation media, where, under favourable conditions, it will be found to correspond very closely with the rapidity of occurrence of the local manifestations in the animal tissues. Anthrax kills very rapidly, and the bacilli grow very rapidly in artificial media at the temperature of the body; whilst tubercle affects locally and gradually, and its organism grows extremely slowly, and only under specially favourable conditions. In twenty-four hours there is a large crop of anthrax organisms, whilst there is usually no evidence of the growth of such a brood of tubercle bacilli until about a fortnight has elapsed.

12. These facts have a most important bearing upon the nature of the processes which are met with in the various forms of disease induced by micro-organisms; and it would be almost possible to draw two lines running parallel to one another, one of which might represent the tubercle bacillus, the other the organism met with in septicæmia, the one acting slowly and locally, the other more rapidly and generally.

Neither organism can develop either its kind or its products if deposited alone on a healthy surface. If, however, sufficient pabulum is carried along with either of these organisms to supply it with nutriment, then the products may be developed in sufficient quantities to devitalise neighbouring tissues, or to neutralise that activity of the cells which prevents their breaking down on the attack of the micro-

organism and its ferment. Both organisms attack a weakened surface or tissue, first locally, the tubercle bacillus acting on the weakened epithelial surface; the septic organism attacking tissues which by bruising or laceration have lost their resisting power. Having once found a coign of vantage, these organisms—though, as seen above, with very different degrees of rapidity—multiply, elaborate ferments, and put to their own use the materials that they obtain from the tissues upon which they are growing. From this point the ferment or product may be absorbed by the lymphatics, and then passed on to the small veins. Here, apparently, the resemblance ceases, though only apparently, for it will be found that in cases of acute tuberculosis, as pointed out by Weigert, there is undoubtedly an invasion of the system by the veins; whilst in certain cases of more chronic septic poisoning the course is readily followed from the original source through the lymphatics, and so to the veins, where the micro-organisms are lost sight of, but are again detected in the secondary inflammatory foci. The difference between the two manifestations could be accounted for by the different rapidity of growth of each of the micro-organisms and its ferment; by the relative diffusibility and activity of the toxic ferment produced; and by the resistance of the tissues to the action of this ferment, either locally or generally. Although such a parallel may be drawn between acute septic poisoning and the tubercular process, there are, nevertheless, certain facts which do not conform to the rule thus laid down. There may be acute septic intoxication without the presence of a single micro-organism in the tissues. Here there is sufficient of the toxic material, developed on a large exposed surface, from which it is absorbed, to give rise to the death of the patient. The alkaloids formed during the decomposition of animal matter, if carefully separated and injected into a vein, or even into the connective tissue, have a similar effect; and even one of the products of the best known form of fermentation—a fermentation which takes place under well defined conditions, and outside the body—alcohol, has a distinct toxic effect on the nerve centres, if taken in sufficient quantities. This toxic effect, though similar in many respects to that induced during the course of the specific infective diseases, must be carefully distinguished from it. In the true specific diseases organisms

are constantly found in relation to the tissues of their hosts, which are in each case typical, and in which they find conditions adapted to their requirements, and favourable to the development of their products.

Selective Affinities.

13. The inquirer into the relations between micro-organisms and disease is met at the very outset by a more difficult question. What peculiarity in the micro-organism itself, or in its host, determines its development or non-development in the tissues of the infected animal? And how is it that micro-organisms appear to exercise a certain selective power, growing readily in one animal, but remaining inactive in another?

Why should the anthrax bacillus attack man and not the pig, an ox or a mouse, but not a dog? Why also should the tubercle bacillus have a special affinity for certain animals, and not for others. How do the house-mouse and field-mouse differ, that in one, "mouse septicæmia" is readily induced, whilst in the other, this is seldom or never the case?

Klein found, as a result of his experiments on swine plague, that he could produce this disease by inoculation in rabbits and mice, but that it is impossible to obtain similar results in man or birds, in the guinea-pig or carnivorous animals.

He further found that infusions of the flesh of any of these groups of animals served equally well as a nutrient material for artificial cultivation of either anthrax bacillus or the bacillus of swine plague. There can therefore be nothing in the dead flesh itself, which, by chemical or other reactions, aids or hinders the growth of the specific micro-organisms. As to the exact nature of the aiding or retarding factors in the living tissues, there are as yet no exact experiments. Anything as yet advanced is mere theory. Klein throws aside the general vital theory, and advances the statement that "the most feasible theory seems to me to be this, that this inhibitory power is due to the *presence of a chemical substance* produced by the living tissue." He therefore specialises the vital theory, but retains it as a means of explaining the chemical. This is as yet incapable of exact proof, but there is a certain amount of evidence in its favour.

The chemical material, it may be assumed, could act in two ways. It might act as a substance without which the microbe could not live, or it might act either as a poison to the microbe, or as an antidote to or a remover of a poison formed by the organism itself under its new condition. In some animals, then, it will altogether inhibit the pathogenetic activity of the organism; in others, where it is not so constituted as to counteract the morbific products, or the activity which gives rise to them, the organism will continue to produce them, and either the cell elements of the host or those of the attacking organism will succumb to their action.

In artificial media it is a frequent experience to find organisms destroyed by the virulence of their own products, before all the food material adapted to their existence has been exhausted; and the clinical evidence of those infective diseases in which a *crisis* occurs (*e.g.*, croupous pneumonia) goes far to support the view that a similar process takes place at times within the body.

14. M. Raulin, in his experiments upon *Aspergillus nigrescens*, found that although it could grow readily on bread moistened with vinegar, it flourished much more luxuriantly on an artificially prepared medium, in which certain definite inorganic materials are present.—(See Raulin's liquid, § 58, p. 110). He found that under ordinary conditions 25 grammes of dried aspergillus is regularly obtained from a definite quantity of the liquid before the whole of the elements are exhausted. If the small proportion of potassium be removed, only one gramme of the dried aspergillus is produced, whilst if the zinc be omitted from the liquid (only $\frac{1}{50.000}$ part of the mixture), the crop gathered amounts to 2·5 grammes only. This zinc is found in small quantities in the aspergillus, and is therefore taken up as one of its aliments. Most minute quantities of nitrate of silver ($\frac{1}{1.500.000}$), of corrosive sublimate ($\frac{1}{50.000}$), of bichloride of platinum ($\frac{1}{8.000}$), &c., act as poisons upon this same aspergillus, and prevent its growth. Duclaux, in commenting upon these facts, points out that "as the plant does not contain any green matter, it may be surprising to see that iron is one of its nutritive elements. Indeed, the withdrawal of that metal produces results similar in importance to those produced by the suppression of zinc. The addition of one gramme of iron to

the nutritive medium will increase the crop by 800 grammes. Notwithstanding this resemblance, the functions of zinc and iron are quite different. Zinc enters the plant as a constituent of its tissues. The only use of iron appears to be to destroy, or suppress pending production, a poison which the plant secretes, and which, were it to accumulate, would end by killing the plant. It is one of those secretions which are common to all living organisms, and whioh they should get rid of at any cost. This is the service iron renders to the aspergillus. Zinc is a physiological aliment; iron is a physiological antidote."

15. Though it be so closely allied morphologically to aspergillus, the *Penicillium glaucum* is found to differ from it in its relations to some of these chemical substances. It is true that it will also flourish in Raulin's fluid, but its growth is rendered more luxuriant by the addition of a little sulphate of lime; and its relations to antiseptic substances differ much from those of the aspergillus, for it can grow in solutions of nitrate of silver and perchloride of mercury, the least trace of which would be instantly fatal to the former. From the close similarity of the functions of the cells of the body and of those of the invading organism, it might have been anticipated that what is food for the one would be food for the other. But, following the analogy just cited, we may infer that this is not the case. One class of nutrient substances will promote the growth of the normal cells, and consequently health; another the growth of parasitic cells, and consequently disease. Similarly one form of medication may destroy the disease, and another the patient.

Mutability of Species.

16. Some first principles are to be grasped before proceeding to the practical points which have to be considered. We have to deal with a confessedly large number of species separated from one another, as it would at first sight appear, by wide differences, both of form and of function, by paying attention to which these species might be arranged and classified. A question has arisen as to how far such an apparently natural classification may be looked on as reliable and

permanent, and it has been held by some that one species of micro-organism may, under appropriate conditions, change its nature completely, and appear in the guise of another species separated from its original form by a wide gap, and this whether the evolution be viewed morphologically or functionally. Such was the doctrine promulgated by v. Naegeli, and still maintained by some of his disciples. But the results of more recent research on this point have rendered such a position apparently untenable, and it has been now pretty generally abandoned as the direct outcome of imperfect methods of research employed in the elucidation of a very difficult, and till recent years a very obscure subject.

Modification of Function.

17. It is, however, by no means inconceivable that in favourable circumstances a micro-organism may change its functional, and even, within certain closely circumscribed limits, its morphological characteristics, in consonance with what is known of the modifications produced by domestication or similarly varied conditions amongst the higher animals; but as in the latter, so also in the former, the thoroughly accredited law of reversion to type comes into play so soon as the abnormal circumstances which occasioned the deviation are removed, and the species once more assumes its typical characteristics.

For this reason, the statements of Buchner and some others have been viewed with grave doubt. They claim to have effected through a long series of gradations a complete change from one well defined species into another, equally well defined, with functions quite distinct from, but morphologically closely allied to, the initial species. They effected this transformation by delicately graduated modifications in the surroundings of the initial species, and, when completed, they regard the change as permanent. At this point, however, it must be distinctly recollected that the long chain of altered conditions thus employed has been in every link exposed to extreme dangers, which even consummate skill as regards *technique* cannot render nugatory; for in every one of the hundreds of re-inoculations carried out in producing the vast number of so-called "generations," between the

commencement of the process and its final issue, there was considerable risk of extraneous contamination. It is nevertheless unjustifiable to urge the extreme difficulty of the quest as the probable cause of a fallacious result; and the position which one must therefore almost of necessity occupy at present is one of expectancy, until more conclusive investigations have been completed, and the whole question put in such a position that its settlement may become a possibility. The evidence on this point so far produced tends strongly against the transmutation theory; but as yet no sufficient mass has been brought forward on either side to render a final decision possible.

Requirements of Micro-Organisms.

18. At this stage it will be well, shortly and in general terms, to state what are the main vital necessities of a micro-organism which determine the relation it will take up to the various tissues or media in which it may occur. Lying as it does on the borderland between the animal and the vegetable worlds, its nature is of a but slightly differentiated type, and its requirements are proportionately simple. An analysis of its chemical constitution shows it to be composed of 88 per cent. of water, 2 per cent. of inorganic ash, and 10 per cent. of organic compounds. This composition gives a general indication as to what substances it will chiefly require to sustain its life and development; and it is found accordingly that its food must consist very largely of watery elements. The organic compounds requisite to its life are chiefly those rich in carbon and nitrogen; and the minerals which it specially needs are potassium and compounds of phosphorus.

Micro-organisms differ to a very great extent as to their dependence upon, or independence of, the presence of free oxygen. So marked is the former condition in certain genera—as, for instance, the moulds—that they do not grow actively except on free surfaces, where they can obtain oxygen directly from the atmosphere, or under such conditions as permit of their readily obtaining it. Other genera, and notably those of the yeast series, are capable of passing through all the stages of their life-history without obtaining oxygen, except the

scanty supply derived by their power of breaking up complex chemical compounds.

These features will require more exact delineation in the section upon the vital functions of micro-organisms.

GERMS.

19. The features of micro-organisms thus far described have applied only to their adult state. Up to the present, but little is known of their germ stage, beyond the fact that their spores are present in water and in soil in innumerable numbers, and are almost constantly floating in the atmosphere as fine impalpable organic dust. Atmospheric germs may even come to have a distinct qualitative value of their own,—not, it is true, from any qualities which they at present possess, beyond what may be summed up in the term "vitality," but in potential properties, which may at any time become active. These possibilities may be roughly gauged by the careful observation of the atmosphere in which they float, as, for instance, noting its hygroscopic conditions—a dry atmosphere being, as a rule, less germ-laden than a saturated one—and such gross meteorological observations as the direction and force of the wind, and the condition of the earth's surface, whether dusty or otherwise. High winds and dust tend to load the atmosphere with germs; absence of wind and rain have the opposite effect, allowing of mechanical subsidence of the floating germs, and providing for their distribution in the soil by the percolating streams of rain, which they perforce accompany. But wherever they may be, they retain their capabilities of growth and development through almost all circumstances short of absolute dessication by heat, or the action of some other powerful germicide, and are prepared to develop into their adult form whenever they are supplied with the necessary conditions of warmth, moisture, and a nutrient soil. The fact that these three factors are so typically present in the case of a surgical wound, accompanied by the lowered vitality of the tissues—also a point of great importance—has led to the development of a special department in surgical practice; and a recognition of the fact that the human body lends such admirable aid to their growth and development is the key to understanding the

significance of medical infective diseases, and the one basis on which a therapeutic system can be constructed. The best researches on the subject lead us to believe that normal healthy tissues are perfectly free from the presence of micro-organisms, and their presence thus directly indicates a diminution in the vitality of the tissue elements near which they lie. Fully vitalised tissues react so powerfully on the micro-organisms, or their germs, as to prevent any development on their part; but this condition once lost, the tissues are no longer resistant, but supply all the requirements of the parasite in the points most closely essential to its life and growth, and become, for the time being, a delicately adapted incubator, in which the life of the organism passes rapidly through its phases.

Conditions necessary to Investigation.

20. In the investigation of any disease where one is led to suspect the interference of a micro-organism, there are certain points of essential importance which must be clearly proved before any such proposition can be established as that a micro-organism is directly concerned in its causation.

(*a.*) Firstly, we must have a well defined type of disease, accompanied in all cases by symptoms so characteristic as to constitute a specific malady. In all such cases, at one stage or other of their course, it must be possible to demonstrate a micro-organism of constant form, and disposed in a constant relation to the blood or solid tissues of its host; and this organism must only be found in the tissues of animals which are suffering, or have suffered, from the distinctive symptoms already observed.

(*b.*) Secondly, by carefully effecting the isolation of these organisms from the tissues, and inoculating them in nutrient media specially adapted for their support, it must be possible to cultivate them artificially apart from the tissues of their host. These cultivations must be absolutely shielded from contamination by the various extraneous conditions with which they may come into relation, and must retain the same typical appearances throughout a long series of cultivations carried on under constant conditions; showing that they have received no new elements from without, nor has any change occurred

within the culture, owing to there having been a commingling of species; for if the latter has been the case, one of the members in this community usually flourishes at the expense of its neighbour, and an alteration in the general type of growth is at once apparent in the artificial cultivation.

By a course of constant re-inoculation through a series of carefully prepared media, any tendency to such an alteration becomes much more prominent; and when a line of demarcation at length becomes clearly visible between two mingled species, the same system allows of their ready separation, and the cultivation of each apart as a single species, such cultures being then termed "pure cultivations."

In certain favourable media, it is possible, by merely noting the salient features, such as the form and general appearance of the growth, to state with certainty not only the fact that it is a pure cultivation, or otherwise, of a micro-organism, but also to place it at once in one or other of the classes into which the lower fungi are divided, and to describe broadly the physiological and pathogenic functions with which such an organism is likely to be endowed.

As Koch points out, these macroscopic appearances and modes of growth must, in the present state of our knowledge of the morphological and physiological distinctions, be looked upon as of even greater value than microscopic and chemical characteristics. Hence the enormous importance attached to the study of these macroscopic appearances, especially in the case of micrococci, whose microscopical differentiation is in many instances absolutely impossible.

(*c.*) In the third place, it must be possible, by the inoculation of the organism thus isolated into the system of an animal liable to the disease, to reproduce it, and the disease so reproduced must have all the symptoms of the original complaint, or at least a sufficient number of them to give it the specific character.

(*d.*) And, fourthly, in the tissues of the animal thus attacked, there must be a recognition of the same foreign elements in the same relation to the tissues of their host as was observed in the original case.

CHAPTER II.

METHODS OF EXAMINATION.

Chemical Reagents.

21. The detection of micro-organisms in fluids and tissues was for long a matter of extreme difficulty, and it has only been during late years that, as the methods of lighting and staining objects were improved, anything like uniform or satisfactory results could be attained.

Without oil immersion objectives with improved condensing and illuminating apparatus, and without aniline colours, the vast field of research now open to mycologists must have remained a *terra incognita*. One can only wonder that so much had been done, when the old methods are compared, or one should rather say contrasted, with those at our command. In this chapter it must be our aim to detail as fully and exactly as possible the various processes by which micro-organisms may be prepared for examination in fluids, and in moderately thick sections of tissues.

Before commencing the description of the methods, it may be well to consider what are the conditions under which these micro-organisms are found, and the different media in which they have to be examined. First, they may be present in some fluid or secretion from the body, as in pus from an abscess, blood, sputum, fluid scraped from a fresh surface of an organ, the fluid drawn from a vesicle or pustule, the secretions or scrapings from the mouth, discharge from the urinary passages, rectum, &c.; in most of which fluids there is a certain per-centage of albuminoid material, a factor which must be borne in mind in connection with the mode of preparation of these fluids for examination. Secondly, it may be necessary to examine certain specially prepared fluids, in which micro-organisms are known to flourish and multiply—fluid cultivating media. Thirdly, they may

have to be examined as they grow on or in some of the solid cultivating media—gelatine, blood serum, bread paste, potatoes, &c.; fourthly, as they occur in the tissues of the body; and lastly, in various organic materials—foods, dust, mould, vegetables, and so on. In whatever position they are found, these micro-organisms react as though they were perfectly free, and the differences of treatment are necessitated, not from any change in the organism itself, but from the different surroundings it has assumed. Thus, it would not be necessary to add any reagent to a cultivation of micro-organisms in a clear meat extract; all the micro-organisms are perfectly distinguishable, and in such a fluid there is nothing else for which they can be mistaken. Alcohol, chloroform, ether, or even strong alkalies or acids, have not the slightest effect on them; there is no alteration in appearance, and the micro-organisms appear as sharply defined, strongly refractile, rounded, ovoid, or rod-shaped bodies, the former arranged in pairs, chains, or masses, the latter most frequently in pairs or chains. On the addition of some of the aniline colouring reagents the micro-organisms in the above fluid stain just as if they were embedded in the tissues or in a mass of sputum.

It is only when they are mixed up with other granular inorganic or organic matter, or when they are embedded in tissue cells, or in intercellular or other spaces in the body, that there is any great difficulty in identifying these micro-organisms, and in assigning to them their proper position in the scale of nature.

These organisms, with a single exception (the *Spirochæte Obermeyeri*), exhibit an extraordinary resistance (optically) to all chemical reagents, for, as noted, they are not changed in the slightest degree by the above-mentioned reagents; and it is to this fact that we owe the possibility of distinguishing them from the tissues or granular material in which they lie, all of which may be more or less altered by one or other of these chemical reagents. The addition of acids will, in most cases, dispose of inorganic granular matter, granular looking fibrin, and even of tissue structure. Ether or chloroform, and alcohol, dissolve out fatty granules or fat crystals. Caustic potash or soda may be used instead of or along with the acid to remove granular material, and to clear up the tissues. The following may be taken as an example of the routine which should be observed

in all cases where the presence of micro-organisms is suspected, whether in fluids or in fresh or alcohol hardened specimens. In the case of fluids, where there is a quantity of albumen present, Baumgarten's method is undoubtedly one of the most effective, and may be applied to any of the fluids, though it is described in connection with the detection and recognition of tubercle bacilli, to which further reference will have to be made.

Baumgarten's Method.

22. Place a small portion of the fluid to be examined on a cover-glass, which has been thoroughly cleaned with nitric acid, and then with distilled water. With another cover-glass press the fluid out into as thin a layer as possible, and wipe the margins with a scrap of blotting-paper. "Separate the cover-glasses by sliding them from one another, when each will be found to be covered on one side with a thin film of sputum."[1] Allow the films so formed to dry, and then, holding one of the covers with a pair of forceps, pass it, "about as quickly as one cuts a slice of bread," three times through the flame of a spirit lamp or a Bunsen's burner in order to coagulate the albumen, which, as already stated, is present in these fluids. Then immerse the cover slips in a solution of a couple of drops of 33 per cent. solution of caustic potash, added to a watch-glass full of distilled water. Press the cover-glass down on a slide, and examine under a high power (× 600), when the bacilli or micrococci may be observed as bright refractile bodies, elongated or rounded, the elongated rods in some cases showing strongly refractile bodies in their substance. It must be remembered, in the case of micrococci, that minute granules of coagulated albumen may be mistaken for these small round organisms, whilst small crystals have been mistaken for bacteria, if this method has not been fully and carefully carried out.

Fresh or spirit-hardened specimens should be treated as follows:— After washing out the gum with warm carbolised distilled water, or getting rid of any embedding material (celloidin, paraffin, &c.) which may have been used, place the sections in absolute alcohol, where

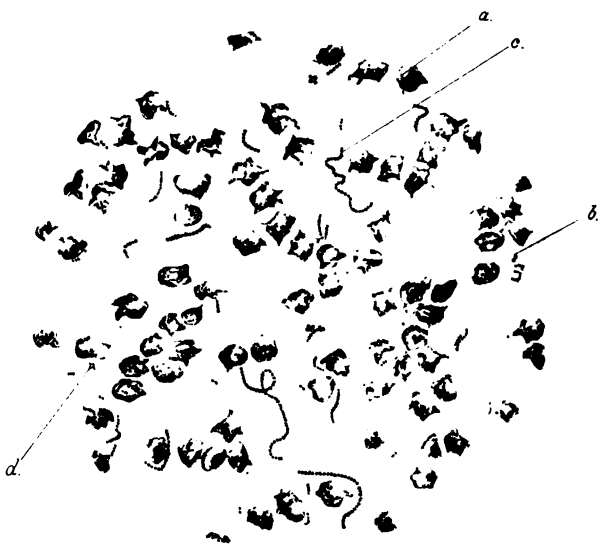
[1] See *Lancet*, 15th July 1882.

they should be allowed to remain for several minutes, then transfer to ether or chloroform in a watch-glass, and afterwards to a strong solution of acetic acid. Wash thoroughly in distilled water, after which the sections may be warmed gently (until bubbles begin to rise) in a two per cent. solution of caustic potash. By this treatment all fat granules, which may be mistaken for micrococci, all small crystals, which are sometimes mistaken for bacilli, granular fibrin, and even differentiation of structure, are removed, and only the resistant groups or strings of micrococci or bacilli are left for examination. Where micro-organisms are present in large masses in vessels or connective-tissue spaces, this method is extremely useful; but for single bacteria it is naturally far from satisfactory. If, however, the single organism is in the form of a bacillus, it may be recognised by its characteristic form, as the only thing for which it can be readily mistaken, the fat crystal, has been removed by the above treatment.

Recognition of Micro-Organisms.

23. In the case of micrococci, the chemical reactions are most important, but there are also certain points to which attention must be paid in regard to evidence of growth, community of form, and size and regularity of arrangement, any or all of which may prove of great service in helping to determine the nature of suspected granules. Most experts agree that the forms of single micro-organisms render but little help to the observer who has to determine their exact nature. But these forms, when considered along with other features, may prove of great value. Thus, when associated with a sharp margin, a well defined outline, a high refractive index—giving it a peculiar glistening appearance—the micro-organism is readily enough recognised. The above refers especially to single cocci, but other characteristics come to our assistance if these small bodies are in groups. For instance, massed together, they have a peculiar brown tinge, and, examining the individual cocci of which this mass is composed, they are found to have all the above characteristics as to form, outline, and appearance; but then another striking feature attracts attention—all the cocci of the same group are of equal size and are of the same form. If, instead of large groups, small numbers of these

cocci come under consideration, it will be observed that there is evidence of their multiplication in their mode of arrangement, and a multiplication which at once removes them from the domain of inorganic nature. They are arranged in pairs (see Fig. 1)—diplococci—as though one had grown from the other by a process of cleavage or partial fission. This process may have been continued so that, instead of a single pair, there is a chain of some length made up of cocci of the same shape and of equal size, the fission taking place in the same plane at each successive division. In other cases the process of fission may take place in planes alternately at right angles to one another, in which event, as pointed out by numerous observers,



J. T. T.

FIG. 1.—Pus from an acute abscess at time of evacuation. Dried and treated with methyl violet. (× 700.)

a. Pus corpuscles between which may be seen the thin film of coagulated albuminoid material.
b. Pair of micrococci—Diplococcus.
c. Chains of micrococci—Streptococci.
d. Sets of four—Tetrads.

peculiar tetrads are formed. *Micrococcus tetragonus* or *Sarcina ventriculi* may be taken as examples of this mode of fission.

In place of either of the above, there may be, as already pointed out, large masses of micrococci, each of these masses embedded in a glue-like matrix—gliacocci. In the case of bacteria there is similar evidence of multiplication in the formation of pairs, where the two elements are often arranged so as to contain a more or less obtuse angle, or in the formation of regular chains—streptobacteria. Most of these points were early recognised, and were described by Hiller, Naegeli, and others. If once it is recognised that the granules grow as above, then they must be organic. There is additional proof of this, as von Recklinghausen, Klebs, Waldeyer, and Friedlaender pointed out, in the fact that in certain cases these micro-organisms continue to multiply, even after death, in the blood-vessels, as is evidenced by the fact that in cases of metastatic pyæmia, ulcerative endocarditis, and similar conditions, small varicose swellings occur at intervals along the course of some of the capillaries and small veins. These dilatations are filled with masses of small round granules which fulfil all the above conditions, and which, by their rapid growth *in situ*, have caused the distention; for only organisms which have this power of multiplication could have brought about such a condition. The same holds good for the distended lymphatics of croupous pneumonia, in which Friedlaender demonstrated the presence of the multiplying micrococci peculiar apparently to that disease.

All bodies which do not conform to the above requirements may be treated as either amorphous or granular precipitates, small particles of inorganic material, or organic molecular granules. Inorganic particles, angular or irregular in form, are also very irregular in their size and mode of arrangement. They are far less refractive than either micrococci or granules of organic matter, whilst by their chemical reaction they are at once recognised. As an example of these inorganic particles may be taken the oxide of iron, which is frequently found in sections stained for tubercle, and then treated with nitric acid. Here the acid acts upon the steel of the needle usually employed, and an oxide is formed, small particles of which are deposited on the section. These are readily enough recognised by one who has worked even a short time at micro-organisms, but they will serve as an example of the case in point. (In all cases where acids are used employ platinum needles.) Organic molecular

granules are not so readily differentiated, but they also may, by careful examination and treatment, be distinguished from micrococci, for which alone they may be mistaken. (Fat crystals may possibly be mistaken for bacilli, from which, however, they may be distinguished by the difference in the manner of arrangement. Bacilli seldom form clusters, but they frequently form strings or chains, the elements of which form angles at their points of junction with each other—see Fig. 2.) The organic granules are more angular, and have a less refractile power than micrococci, though they are more regular in form and more refractive than inorganic particles. The colour is variable, whilst the size and grouping are always more or less irregular.

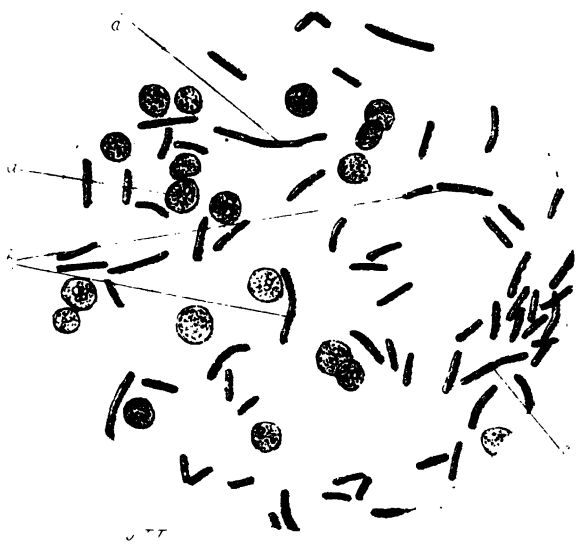


FIG. 2.—Anthrax bacillus in scraping taken from cut surface of the spleen of a cow, in a case of splenic fever. Specimen dried, then stained with Bismarck brown, and mounted in a solution of acetate of potash. (× 700.)

a. Small corpuscles from spleen.

b. Bacilli. Some, simply short rods; others, made up of a couple of rods which enclose an angle; others again, arranged in long threads. The bright spores are to be seen in some of these chains (*c*), whilst fission is taking place in others (*d*).

Here, too, the chemical reactions are invaluable, and, by the aid of ether, chloroform, liquor potassæ, acetic acid, &c., fat globules, com-

pound granular corpuscles, caseine, slender filaments of fibrin (Cohn), or fibrin becoming granular, may in turn be distinguished from the more resistant micrococci. In tissues it is a good rule, if no other chemicals or staining reagents are at hand, and where the presence of masses of micrococci is suspected, to macerate the sections for an hour in a ten per cent. solution of liquor potassæ, and then stain with a solution of iodine; by this method the bacteria are stained brown, whilst fat granules, &c. remain uncoloured.

Staining Reagents.

24. It is at once evident that, by careful attention to details such as the above, micro-organisms can be distinguished with tolerable certainty; but it is only by calling to our aid staining methods that we are able to study the minute structural peculiarities of these organisms; in fact some of the most important peculiarities yet recognised come out only as chemical colour reactions. Staining methods, as applicable to the study of micro-organisms, are daily becoming more perfect, and though in some cases they are being elaborated, the general tendency is undoubtedly towards a simplification of the processes. To Weigert and Koch we are especially indebted for most of the elegant methods now at our command, —methods which are based on the use of aniline colours.

It may be laid down as a general statement that micro-organisms react to staining fluids very much as do the nuclei of cells. This holds good so far, that most nucleus tinting materials will impart a similar tint to micro-organisms; beyond this, however, it is found that the micro-organisms hold the colouring matter more tenaciously than do the cell-nuclei, and that the nuclei may be decolorised by the use of certain reagents, such as carbonate of potash, recommended by Koch, acetic acid, or one of the mineral acids, nitric or hydrochloric.

In this connection it must be remembered that many micro-organisms can at present only be classified according to their reactions with the colouring and some one of the various decolorising reagents. Some take on one stain more readily and retain it more persistently than any other, and some, which in the first instance take up the

same colour, yield it much more easily to one reagent than to another In working with unknown bacteria, therefore, it is necessary to experiment in turn with the various colours, and then the various alkaline and acid decolorising reagents, and to note the reaction in each case. A certain amount of discretion should also be used in the after treatment of stained specimens, for although some of the aniline colours are only slightly soluble in clove oil, others are extremely soluble, though they are, in turn, unacted upon by turpentine, xylol, oil of bergamot, or cedar oil. Even the solvent for the Canada balsam should be a matter for careful consideration. Chloroform is perhaps the worst of all. When aniline stained specimens are to be mounted, its place should be taken by turpentine, benzole, and xylol, or the balsam should be simply warmed and used without any solvent. These matters appear trifling in themselves, but upon a discriminating carrying out of them depends the success of the operator in obtaining permanent and reliable preparations.

Flügge,[1] on the authority of Ehrlich, classifies the aniline colours into two distinct groups, each of which has very distinct chemical and physiological characteristics,—the acid and the basic dyes.

In the first group the colouring matter acts as an acid in combining with bases to form salts, although it does not necessarily give an acid reaction, nor is it necessarily in the form of a free acid. Amongst the more important are eosin, picric acid, aniline black, alizarin, purpurin, and perhaps hæmatoxylin.

To the second group—the basic dyes—belong by far the greater number of those which are used in staining bacteria. These are fuchsin, rosaniline, methyl violet, methyl green, Magdala red, and especially Bismarck brown, dahlia, and gentian violet. These basic colours are sold as salts, and not as free bases, whilst fuchsin, for example, may be sold as an acid salt, as chloride or acetate of rosaniline.

With these nucleus and germ tinting reagents we can colour both nuclei and bacilli :—

Red with nucleus tinting carmine solutions, with fuchsin, magenta, &c.

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[1] "Handbuch der Hygiene," Pt. I., *Fermente und Microparasiten*, C. Flügge, Leipzig, 1883, p. 287.

Brown with Bismarck brown, vesuvin, and chrysoidin.

Green with methyl green.

Blue or *violet* with hæmatoxylin, methyl blue, methyl violet, dahlia, gentian violet, &c.

The first experiments on staining germs were made with carmine and hæmatoxylin, and Koch and others were successful in staining not only the bodies of bacteria, but also, in certain cases, in demonstrating the flagella. These reagents are now, however, superseded by the aniline colours, principally the basic series (though these will not stain flagella of bacteria as well as a concentrated aqueous extract of logwood, the specimen being afterwards treated with a solution of chromic acid).

Weigert's gentian violet and picro-carmine method demonstrates

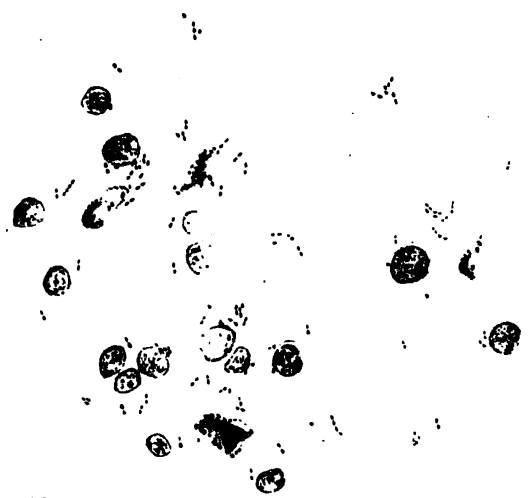

FIG. 3.—Blood taken from a case of traumatic tetanus. Blood corpuscles and micro-organisms stained with methyl violet, mounted in Canada balsam. (× 700.)

most admirably the affinity of the basic aniline colours. The sections are first placed in gentian violet (§ 27, p. 40), then washed in alcohol,

transferred to water, and afterwards to Weigert's solution of picro-carmine for half an hour.

They are further washed first in water, then in alcohol, are clarified with clove oil, and mounted in balsam. By this method the nuclei are stained red and the bacilli violet; the carmine has replaced the gentian violet in the nuclei, but has had no effect upon the violet taken up by the bacilli. In a similar manner, a solution of iodine and iodide of potassium does not remove the basic colours from micro-organisms, but it rapidly decolorises nuclei and other tissues. It is upon this fact that Gram's method is founded.

For staining most bacilli and micrococci a saturated watery solution

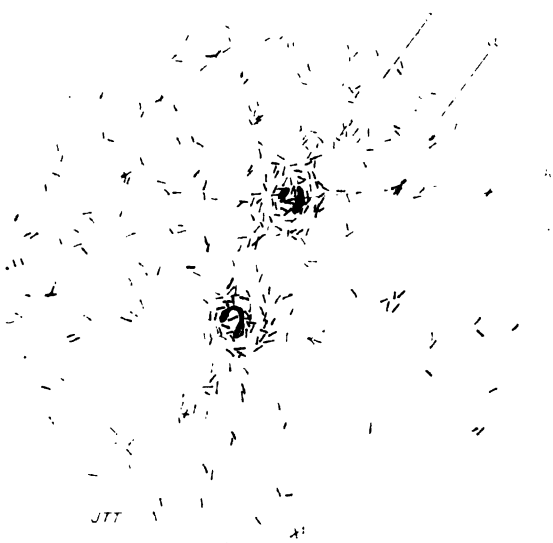

FIG. 4.—Blood of a mouse killed by mouse-septicæmia. Blood dried, heated, and stained with methyl blue, and mounted in glycerine. (× 700.)

a. White blood corpuscle with horse-shoe-shaped nucleus, and numerous minute bacilli in and around it.

b. Red blood corpuscles.

c. Small bacilli between corpuscles.

of gentian violet or methyl violet (ten minutes), methyl blue (thirty minutes), or Bismarck brown (twenty-four hours) may be used. The

time required for the section to become stained varies also with the temperature, and the above periods may be considerably shortened if the temperature be slightly raised. When sufficiently deeply stained, the sections are washed in distilled water, then in weak acetic acid, and again in distilled water. If the colour is to be allowed to remain in the nuclei, the section should be mounted at once in Canada balsam, dammar varnish, Farrant's solution, glycerine jelly (prepared by dissolving one part of gelatine in six parts of water, adding seven parts of glycerine and one per cent. of carbolic acid; heating the whole, and then filtering whilst warm), a saturated solution of acetate of potash; or for Bismarck brown preparations, glycerine.

Where alcohol and clove oil are used in the process of mounting, great care should be taken that the sections are not allowed to remain too long in either of these fluids, or by their continued solvent action even the dyes in the bacteria may be partially or wholly removed, if not at first, after the lapse of some time. If the nuclei are to remain unstained, and the sections are to be cleared up as much as possible, the section is stained, afterwards washed in distilled water, and then transferred to a five per cent. solution of carbonate of potash, by which the colouring matter is discharged from all the tissues except the bacilli or micrococci. This method is invaluable for the demonstration and enumeration of bacilli contained within vessels or thick sections, as in intestinal mycosis, where the anthrax bacilli are to be observed *in situ* in the capillary vessels of the intestinal villi.

In place of a watery solution, a saturated alcoholic solution of almost any of the germ-tinting aniline colours may be made up in large quantity, and diluted as required with about ten times its bulk of distilled water or of aniline oil water.—(Weigert.)

GRAM'S METHOD OF STAINING.

25. Perhaps one of the most satisfactory methods is that recommended by Gram. All specimens to be stained by this method should be preserved in absolute alcohol, and should be transferred at once to Weigert's or Ehrlich's gentian violet, or fuchsin and aniline water solution (§ 27, p. 40), where they may remain for from one to three

minutes (tubercle sections should be left for from twelve to twenty-four hours), wash in alcohol for three minutes, and then in a solution of ten parts iodine, twenty parts iodide of potassium, and 3000 parts of water, until the dark blue violet is replaced by a dark purple red. Wash in alcohol until most of the colour has disappeared, then clear up in oil of cloves until still more of the colour is removed. The sections may be mounted at once in balsam when the tissues and nuclei have a faint yellow tinge, and the micro-organisms are deep blue or almost black; or they may be stained with a deeper contrast stain, such as an alcoholic solution of eosin or Bismarck brown, or a

FIG. 5.—*Bacillus anthracis.* Stained by Gram's method with methyl violet and vesuvin. Mounted in Canada balsam. (× 700.) Anthrax rods and filaments stained with the methyl violet. Some spores are seen as bright points in the rods. Cells from pulp of spleen of cow from which specimen was taken are stained brown by the vesuvin.

watery solution of vesuvin. Cover-glasses, with their films of sputum, &c., after being prepared (see § 22, p. 26) are treated in exactly the same manner as sections.

Stains for Special Bacilli.

26. Most of the known micro-organisms may be stained by the above method, but there are some which require special treatment, and there are probably others for which a method of staining has not yet been discovered. For instance, the glanders bacillus is best stained by an alkaline solution of methyl blue, which, according to Schütz, is prepared by making a one-tenth per thousand watery solution of caustic potash, and adding one-third the bulk of a saturated alcoholic solution of methyl blue. The sections are then treated

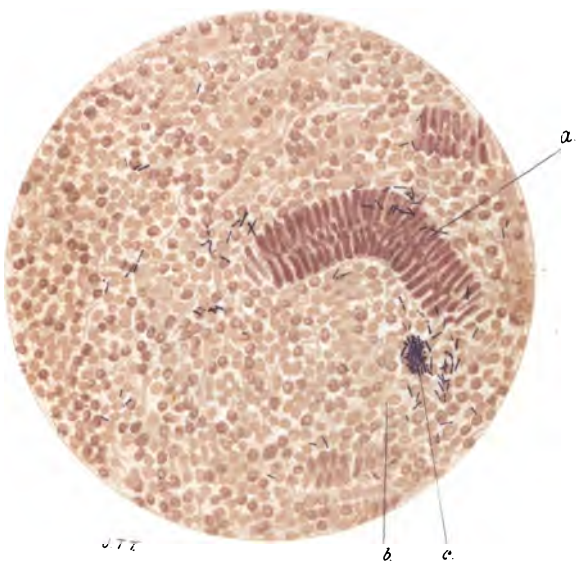

Fig. 6.—Bacilli in swollen mucous membrane of lower part of small intestine. Case of typhoid fever, child. Stained by Gram's method, gentian violet and eosin. (× 300.)

a. Epithelium of crypts.

b. Small round cells like adenoid corpuscles, amongst which the violet stained bacilli (*c*) are found in enormous numbers. Here and there are a few larger endothelioid cells.

with dilute acetic acid, and mounted in some of the various mounting media.

Typhoid bacilli are also stained by Schütz's methylene blue solu-

tion, but not so well by Gram's method, unless they have been soaked for a few minutes in a strong (one per cent.) solution of bichloride of mercury after they are stained with the gentian violet, when extremely good results are obtained. Gram considers that the bichloride of mercury acts as a mordant, or fixer of the colour, in the bacilli.

The bacillus of leprosy also requires special treatment; it is perhaps most readily stained by Koch's original tubercle stain, alkaline

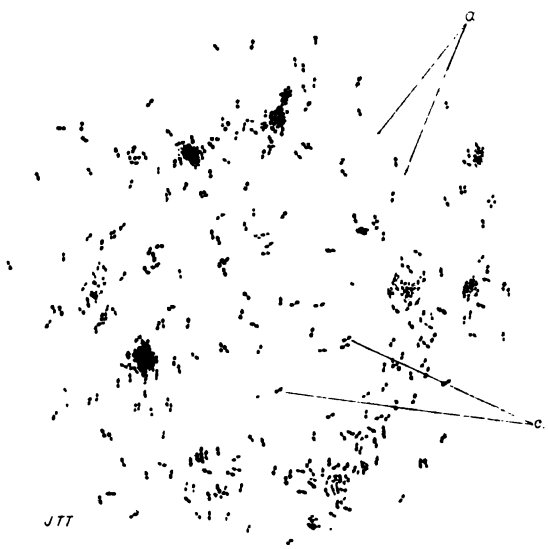

FIG. 7.—Micrococci from sputum in a case of acute pneumonia during the early stage. Stained with gentian violet and eosin. (× 700.)

a. Pus corpuscles, stained by eosin.

c. Micrococci, with delicately stained capsule.

methyl blue, with a watery solution of vesuvin for the contrast stain. Methyl blue and fuchsin are also recommended.[1]

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[1] *Lancet*, August 23, 1884. "*The Demonstration of the* Bacillus lepræ.—Dr. Manson gives a most admirable method of demonstrating, for diagnostic purposes, the presence of the *Bacillus leprœ* in the infiltrated leprous patches. 'A leper tubercle or infiltrated patch is selected, and the whole or part of it included in the jaws of an ordinary thin-bladed pile clamp. The tightening of

For certain organisms, micrococcus of pneumonia in sputum, micrococcus of gonorrhœa, &c., Klein recommends a mixture of methyl blue and vesuvin. Gram's method appears, however, to be preferable for the micrococcus of pneumonia, whether in sputum or in the tissues.

Friedlaender succeeded in staining the pneumonia micrococcus in the tissues by the following method. Stain the sections in

Fuchsin, ...	1 part.
Distilled water, . . .	100 parts.
Alcohol, ...	5 parts.
Glacial acetic acid, . . .	2 parts.

Afterwards rinse them first in alcohol and then in a two per cent. solution of acetic acid, clear up in alcohol and oil of cloves, and mount in balsam.

METHODS OF STAINING TUBERCLE BACILLI.

27. The tubercle bacillus differs from most other bacteria in the fact that it holds the aniline stains with great tenacity, even when acted upon for a short time by a strong mineral acid, or for a longer time by a dilute solution of one of these acids.

The most favourable results are obtained if the thin layer of sputum or thin section of tissue be first treated with some alkali such as potash lye, or, better still, aniline oil. In place of an alkali, carbolic acid one to twenty has been used, and with some success, and even a one per cent. solution of bichloride of mercury.

Koch's original method was as follows :—

The cover-glass with the dried and coagulated film (§ 22, p. 26) is

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the clamp has the effect of driving out all the blood from the included tissues, and the tubercle, from being dirty red, or purple, becomes like yellow wax. The hold of the clamp is maintained at a degree of tightness sufficient to keep up this state of anæmia, and at the same time the centre of the included mass is pricked with a needle or sharp knife. From the puncture a droplet of perfectly clear fluid exudes, and is to be transferred to one or more cover-glasses, each cover-glass being smeared with rather a thick layer of the leper juice. The cover-glasses are then dried, stained, washed, and mounted in the ordinary way. The Weigert-Ehrlich method I have found gives good results. Under the microscope, slides so prepared show bacilli in prodigious numbers, both free and in dense bundles, packing the leper cells.' Klein recommends that the film be stained with magenta, washed in distilled water, and then stained with methyl blue."

placed in the following solution for from twelve to twenty-four hours :—

Methyl blue concentrated alcoholic solution, .	1 part.
Distilled water, ...	200 parts.
10 per cent. caustic potash solution, . .	2 parts.

Stir this mixture well and frequently.

Then immerse the cover-glass for a couple of minutes in a watery solution of vesuvin, until the blue coloration has disappeared, rinse with distilled water, examine at once, or allow the film to dry thoroughly, and then mount in Canada balsam. Sections may be cleared up with clove oil or cedar oil, as recommended by Koch.

By this method the tubercle bacilli are stained blue, and the rest of the tissues, pus corpuscles, &c. brown. Ehrlich sought to simplify the process, or rather to render the detection of the bacilli more certain, by using one of the mineral acids as a decoloriser, having found by experiment that the acid had no effect upon the rods stained with gentian violet or fuchsin, although other tissues were rendered colourless. Koch very early adopted Ehrlich's plan, which is as follows, as given by Koch.[1]

Stain sections of tissues or films on cover-glasses for at least twelve hours in the following solution :—

Saturated alcoholic solution of methyl violet or fuchsin,	11 parts.
Aniline water, ...	100 parts.
Absolute alcohol, ...	10 parts.

The staining of films on cover-glasses may be accelerated by gently heating the staining solution until steam rises from the surface. Such preparations are stained in from a quarter to half an hour under these conditions. Pass the preparations rapidly through a dilute (one to three) watery solution of nitric acid, care being taken that this is perfectly free from nitrous acid, that sections are never allowed to remain more than a few minutes—two or three—and cover-glasses half a minute. Any colour that remains after this is perfectly soluble in sixty per cent. alcohol, but it is by no means so soluble in water, hence the preparations are

[1] R. Koch, "Die Ætiologie der Tuberkulose:" *Mattheil. aus dem Kaiserl. Gesundheitsamte*, vol. ii.

at once transferred to alcohol of such a strength, and left there for ten or fifteen minutes, after which they are stained for several minutes with a weak solution of vesuvin or methyl blue. Rinse with sixty per cent. alcohol, and then with absolute alcohol, as for ordinary balsam specimens. In the further preparation of these specimens, Koch objects strongly to the use of clove oil for clearing up the preparations, and advises the use of turpentine or cedar oil, neither of which removes the aniline colour from the specimen. He also advises the use of turpentine as a solvent for the balsam. In preparations treated in this manner, before the second staining

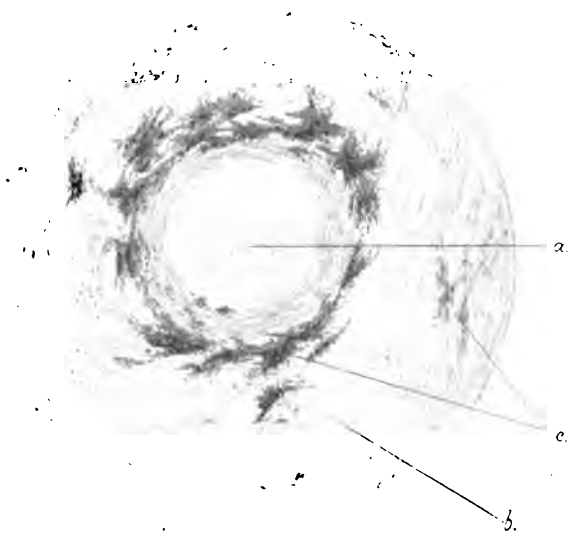

FIG. 8.—Section of liver. Acute tubercle. Stained with gentian violet. No contrast stain. (× 400.)

a. Section of a small vessel in the portal space.
b. Connective tissue of portal space.
c. Enormous masses of tubercle bacilli in the perivascular lymphatics, and also in the lymphatics or connective tissue spaces.

Some of the bacilli have been carried into the lumen of the vessel, where they are in contact with the blood corpuscles. This is probably accidental.

there is not the slightest coloration of the tissues, or at most there is a faint blue tinge, but the tubercle bacilli stand out very pro-

minently as deeply stained rod-shaped bodies. It is, however, extremely difficult to observe the relations of these rod-shaped bodies to the tissues in which they lie, especially when they are present in small numbers, or where they occur as single rods scattered at great intervals. In order to obviate this difficulty, a second or contrast colour is used, a colour which will stain the nuclei and cells deeply, and which will also serve as a background to bring into relief the bacilli.

If the bacilli are stained with methyl blue or gentian violet,

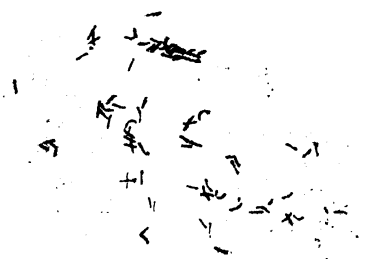

FIG. 9.—Tubercle bacilli in sputa. Stained with gentian violet. Contrast stain Bismarck brown. Weigert's method. (× 450.)

Bismark brown or vesuvin forms the best contrast stain; with magenta or fuchsin methyl blue is preferable.

Since Weigert, Ehrlich, and Koch worked at these methods, there have been innumerable modifications, most of which have for their object the simplification and shortening of the process, and are specially devised for the use of clinicians.

28. *Rindfleisch's method.*—First prepare a solution as follows:—Saturated alcoholic solution of fuchsin, ten drops; aniline water, freshly prepared and filtered, two drachms. Pour this into a watch-glass, and place the cover-glass prepared face downwards on the surface; with a pair of forceps hold the watch-glass over a spirit lamp or other flame until steam rises from the surface of the staining fluid. Remove the cover-glass with a pair of forceps; wash off the super-

fluous stain with distilled water, and then transfer to a watch-glass half filled with alcohol, to which has been added a couple of drops

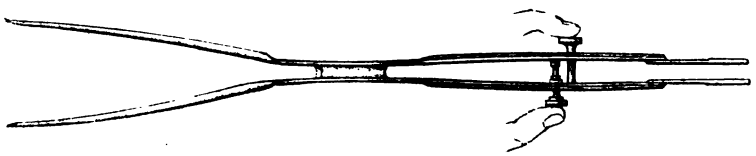

FIG. 10.—Pair of spring forceps with platinum points. These are so constructed that a cover-glass is held between the points when no pressure is exerted. The other end is simply a pair of ordinary forceps.

of nitric acid; allow it to remain here for about a quarter of a minute; again wash in distilled water; dry, and mount in balsam. Here of course only the bacilli are stained, but a contrast stain may be used, if it is thought necessary.

29. *Kaatzer's method.*—A capital method is that recommended by Kaatzer, who, after staining the prepared film on the cover-glasses with aniline water, 10 c.c., and saturated alcoholic gentian violet solution fifteen drops, warmed to about 80° C. for a few minutes, and removing the superfluous staining fluid with clean blotting-paper, immerses them for about a half to one minute in a solution made up as follows:—

Alcohol, ninety per cent. . . .	150 parts.
Distilled water, ...	30 parts.
Hydrochloric acid, . . .	1 part.

Wash thoroughly in ninety per cent. alcohol (one to two minutes) until the whole of the colouring matter disappears from the film; allow to dry, and then drop on the prepared surface a few drops of a concentrated watery solution of vesuvin. At the end of a couple of minutes again wash in distilled water, dry, and mount in balsam. This method gives exceedingly good, and, according to the author, very lasting results.

Heneage Gibbes' modification may also be used, especially where

a rapid double staining of sputum is desirable. He uses the following mixture:—

Rosaniline hydrochloride,	2 parts.
Methyl blue,	1 part.
Triturate in a glass mortar.	
Then dissolve aniline oil,	3 parts.
In rectified spirit,	15 parts.
And slowly add distilled water,	15 parts.

Keep in a stoppered bottle; allow the cover-glass with the hardened film of sputum to remain in this solution (warmed) for four or five minutes, and then wash in methylated spirit until no more colour comes away; drain thoroughly, and dry. Sections may be stained in the same fluid in three or four hours.. Mount in Canada balsam.

Each of the above methods has its own adherents, but the most satisfactory results are obtained by Ehrlich's method, as modified by Koch and Kaatzer.

Sources of Error in Observation.

30. In examining stained specimens, the difficulties as regards the identification of the various micro-organisms are to a very great extent removed, but the beginner will, through imperfect staining and inaccurate observation, undoubtedly be led into error, if it be not borne in mind that, unless special methods of treatment be adopted and carried out in all their details, the nuclei or parts of the nuclei retain the germ-tinting reagents. In a great number of cases these nuclei appeared to be greatly broken up, and in such cases it is an extremely easy matter to mistake the fragments of the nucleus for a mass of micrococci in the centre of the cell.

Compound granular corpuscles, the small rounded masses of fatty granules that are so frequently met with in tissues undergoing fatty degeneration, are also not unfrequently mistaken for masses of micrococci, even in fairly well stained specimens, and albuminoid granules or other amorphous particles are similarly mistaken for stained micrococci.

Such mistakes, however, cannot be often repeated, and after very

little practice and careful observation, such errors may be left out of court entirely.

Examination of "Fresh" Preparations.

31. Although up to the present we have considered the examination of dried and stained specimens only, it will be readily understood that, after all, such examinations are far from perfect, and that the appearances during life and the chemical peculiarities can only be determined by a careful examination of the micro-organisms in the fluids or tissues in which they are growing. Such an examination should be made in all cases where motion of the object in question could afford us any clue as to its nature. In blood, for instance, in which septic bacilli (Pasteur) are supposed to be present, the *spontaneous* movements of the bacillus will afford a most direct proof of its vitality. By these spontaneous movements currents are set up, and shifting of the positions of the corpuscles are brought about, even when the organisms themselves are distinguishable only by the exercise of the greatest care.

Such movements are of even greater importance when we come to the examination of bacteria cultivated in fluid media, for it is by these that the organic nature of the growth in the fluid may be at once determined. At the same time, as Naegeli points out, the movement should be spontaneous to be of any very great diagnostic value, and should be sharply differentiated from the oscillatory Brownian movements, or from those movements brought about by currents caused by evaporation, altered temperature, movements of the slide or cover-glass, and other similar external causes.

By far the simplest method for such fresh examination is to take a drop of the germ-laden fluid, blood, meat broth, sugar solution, &c. in a fine glass pipette, place it on a clean slide, put on a cover-glass, and examine at once. The fluid is perfectly transparent, and nothing but the well defined micro-organisms are seen, either alone or between the blood corpuscles, or in or around other cells. Should there be any tendency to dry, a drop of distilled water, or, still better, a drop of three-quarter per cent. solution of common salt, that has been thoroughly sterilised by boiling, may be added. By dropping such

fluids at the margin of the cover-glass, micro-organisms may be kept unaltered and under observation for a considerable period. Small fragments of tissue may be pressed out or teased out in the same fluids, but never in the serum which exudes from the neighbourhood of the incision, for the fluid itself may contain numerous micro-organisms, which, becoming attached to, or lying on, or under the fragments, may give rise to appearances which would otherwise not be seen, and are therefore often very misleading.

Cultivations on solid media, peptonised meat jelly, agar-agar, Koch's blood serum, &c., should always in the first instance be treated in the same way, by squeezing out between the cover-glass and slide, and then by adding distilled water or the saline solution.

Where such specimens are to be kept for comparison or further observation, a ten per cent. solution of acetate of potash forms a capital mounting medium; or, in place of this, a mixture of glycerine and water, or Farrant's mounting fluid, may be used.

In this fresh condition the various chemical reagents (§ 21, p. 25) are used to great advantage, and the reactions are exceedingly well defined

His's Method of Staining and Washing.

32. In many cases it is necessary to stain micro-organisms, as they exist in fluids which contain no albumen, and in which there is nothing that by the application of heat, alcohol, or bichloride of mercury can be rendered insoluble, and which, therefore, have to be stained and washed in such a manner that they are not removed from the slide or cover-glass during the process. His's method of applying the staining fluid at one edge of the cover-glass under which is the material to be stained, and setting up a slight current by sucking up fluid at the opposite margin with a piece of clean blotting-paper, is the best for this purpose. The specimen is washed first with water, and then with alcohol in the same manner, after which it may be mounted in glycerine, run in at the margin; or the glasses are separated, the films are allowed to dry, and then mounted in Canada balsam. The best stains to use with this method are methyl blue and Bismarck brown or vesuvin.

Fresh sections may be treated as already described (§ 31, p. 45), but it is well to pass them through a saturated solution of bichloride

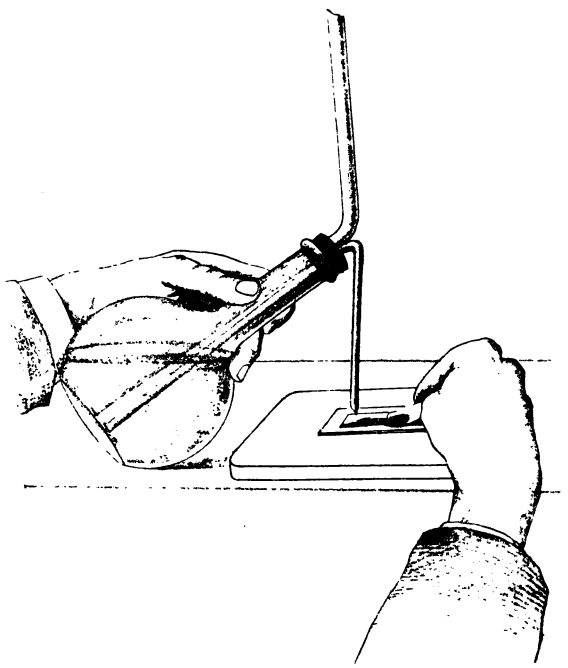

FIG. 11.—His's method of washing off extra stain from dried films in which there is no coagulable albumen. See description in text.

of mercury, by which the protoplasm of the tissues is fixed; they then may be transferred to a three-quarter per cent. saline solution, and stained and mounted.

HARDENING OF TISSUES.

33. Although such examination in the fresh condition should always be made where possible, the results obtained are never so satisfactory as where the tissues in which the micro-organisms are embedded are slowly and carefully "fixed," and where a more deliberate examination may be made.

Koch insists that all tissues in which the presence of micro-organisms is suspected should be hardened in large quantities of absolute alcohol. Such a method, however, is extremely expensive, and, except for small pieces of tissue, which should be kept as test pieces, is scarcely necessary, as methylated spirit, or even a mixture of two parts Müller's fluid and one part of a two per cent. solution of carbolic acid appears to answer equally well. In putting away tissues to harden, the pieces should never be more than about half an inch or three-quarters of an inch in diameter; and where absolute alcohol is used they should be even smaller, otherwise the centre of the cube is untouched by the reagent, which hardens very rapidly indeed (within a week). The other fluids harden the pieces in from two to five weeks. Tissues hardened in Müller's fluid and carbolic acid should be transferred to methylated spirit at the end of five or six weeks, after being washed in distilled water for twenty-four hours. All sections, whether of fresh or of hardened tissues, should be thoroughly washed in absolute alcohol before they are stained (Gram), and sections to be kept for any length of time before they are mounted are best preserved in the same medium.

Method of Illumination.

34. As some of the organisms to be examined are exceedingly minute, it is only by the greatest care and with the aid of the most perfect illuminating apparatus that many of them can be recognised, and when their presence is recognised, their position in the tissues can often be determined only by carefully modifying the light and other conditions. The recognised combination in Germany is Zeiss' $\frac{1}{12}$th inch oil immersion lens and an Abbe's illuminator, and this undoubtedly combines most of the requisite conditions. The condenser has a very short focus, and must therefore be brought as near to the under surface of the glass as possible. It may then be used as an ordinary condenser if the lateral or greatly converged rays be cut off by means of a diaphragm with a small aperture, placed below. If a large aperture in the diaphragm be used, the greatly converged rays of light are also allowed to play on the structures, which are lighted from all points, all shadows disappear, and the "structural

picture" is lost. As the structural picture is lost, however, the stained elements, such as the micro-organisms and the nuclei, are brought out prominently and can be carefully examined. All preparations should be examined with both the small and the large apertures beneath the condenser. With the small aperture, the structural elements are observed and the positions of the organisms; as the apertures are increased in size, the organisms become more and more prominent, whilst the structural elements gradually disappear from view.[1] Even when no micro-organisms can be found in fluids and tissues examined under ordinary conditions, they may still be present and may be brought into view by the use of the above optical combination.

Messrs. Powell & Lealand make an equally good combination, some authorities even preferring their $\frac{1}{12}$th in oil immersion to Zeiss' glass.[2] They also make a $\frac{1}{25}$th in oil immersion which is an excellent glass, but such a power is of comparatively little use except for very special work.

PHOTOGRAPHY.

35. Photography is now in many cases called to the aid of the observer, and to help to record with accuracy what can be seen under the microscope. Koch, by his admirable series of photo-micrographs, has proved that most valuable results may be obtained by this method, and that features which may escape observation under the microscope come out prominently in a photograph, especially in enlargements. He uses wet or collodion plates, hence his apparatus, consisting of Heliostat, condensers and the rest, is somewhat complicated. For the ordinary worker the dry plate process gives sufficiently good results, and with much less trouble and far simpler apparatus. Koch works without an eye-piece, but this appears to be a matter of detail, as many workers prefer to use it. We have found that with an oxy-hydrogen light, or even with an incandescent electric lamp, it is perfectly easy to use the eye-piece and a $\frac{1}{12}$th inch objective (Zeiss' homogeneous immersion), with which arrangement it is found practicable to have a comparatively short camera, and the necessity

[1] "Traumatic Infective Diseases," Koch, 1880. (German, 1878.)

[2] "Practical Histology and Pathology," Heneage Gibbes, 2nd ed., 1884.

for any adjustment, except that on the microscope, is done away with. Even an ordinary argand gas-burner gives sufficient light for systems magnifying up to about 400 diameters.

In working with high powers it is necessary to concentrate the light as much as possible, for which purpose two condensers are used—one to bring the rays parallel, and a second to bring them to a focus on the mirror, or directly on the specimen.

All that is necessary is a flat board, to which the camera is screwed (an ordinary wooden camera is quite good enough). Around the aperture in the front of the camera is a cloth tube with an elastic ring, which fits over the eye-piece of the microscope. The microscope is clamped by means of thumb-screws to a piece of board firmly fixed at right angles to that on which the camera rests, and so far away that when the tube is pushed in it leaves the projecting ring or shoulder of the eye-piece tube within the elastic band. This apparatus may be used in a horizontal position, where the specimens to be photographed are firmly fixed, as in the case of tissues mounted in balsam, in which position the light is most readily managed, but by simply having a couple of hooks at the camera end of the board, the whole apparatus may be hung against a wall, and the slide is kept perfectly horizontal, in which position fluids or unfixed specimens may be easily photographed. One great advantage connected with this apparatus is that as the specimen can be searched, and the exact point to be photographed fixed before the microscope is clamped to the foot-board, no mechanical stage is necessary. The best photographs are obtained of specimens that have been stained brown, hence, as Koch suggests, Bismarck brown, vesuvin, or chrysoidin should be used as the staining reagents. Fairly good results, however, are frequently obtained from unstained preparations, and even from preparations stained with eosin, or others of the aniline colours; and as certain micro-organisms can only be stained with actinic colours, contrasts must often be called to our aid, double-staining being especially useful in such cases.

The mounting reagent must be as free from colour (yellow) as possible; this is of even greater importance than absolute transparency. The best medium is a saturated solution of acetate of potash; after

this come glycerine and dammar varnish, but Canada balsam, which has almost invariably a yellow tinge, should be absolutely tabooed.

Apparatus and Reagents.

36. In addition to the ordinary apparatus and reagents, the following should always be kept near at hand and ready for use :—

Clean cover-glasses.

A couple of platinum needles (Fig. 21).

A pair of platinum-pointed forceps (Fig. 10).

A Bunsen's burner, or a spirit lamp.

A number of watch-glasses and shallow glass dishes.

A white porcelain slab or tile.

A large flat dish—glass or porcelain.

A couple of wide-mouthed glass stoppered bottles; one containing caustic potash, the other containing turpentine. Old slides and cover-glasses are transferred to these fluids, one or other of which will dissolve most of the mounting reagents.

A wash bottle containing distilled water.

FIG. 12.—Small narrow-mouthed bottle fitted with funnel filter, used especially for aniline staining fluids and aniline water.

A number of small glass funnels and filter papers.

Plenty of white filter or blotting paper.

Test-tubes and rack.

Bottles (Fig. 12) to hold stains. Each bottle has, instead of a stopper or cork, a small glass funnel and filter paper, through

which the fluid may be filtered, and which serves to keep out dust from the bottle.

Three or four small filter ring-stands.

A large bottle of three-quarter per cent. salt solution, which has been carefully boiled.

Acetic acid; one in four.

Glacial acetic acid.

Nitric acid, pure.

Hydrochloric acid, pure.

Carbonate of potash solution.

Caustic potash solution, forty per cent.

Strong ammonia.

Ether.

Chloroform.

Alcohol, methylated.

Alcohol, anhydrous.

Perchloride of mercury solution, one per cent.

Saturated or ten per cent. solution of acetate of potash.

Osmic acid, half per cent., kept in a wide-mouthed bottle covered with brown paper.

A saturated solution of aniline oil in water. Prepared as follows:—

Aniline oil, ...	1 part.
Distilled water, . . .	3 parts.

Shake well every half hour for three or four hours, and decant the water as the oil settles to the bottom. The commercial aniline may be used instead of the pure form, as it is only about one-twelfth of the price, and answers almost as well.

Watery solutions of the aniline dyes, especially gentian violet, methyl violet, methyl blue, chrysoidin, vesuvin, and magenta. Any of these may be used as a half to two per cent. solution. They should be kept in the filter bottles, in each of which a crystal of thymol should be placed.

Saturated alcoholic solutions of gentian violet, fuchsin, eosin, and other dyes, if necessary. These should be kept in well stoppered bottles, but should be filtered before they are used. A crystal of thymol should be added.

Weigert's picro-carmine solution (Grübler, Leipzig).

Bismarck brown.

(*a.*) Dissolved in equal parts of glycerine and alcohol.

(*b.*) Dissolved in glycerine, two to four per cent. solution.

(*c.*) Aniline brown, ... 1 part.
Alcohol, anhydrous, . . . 10 parts.
Distilled water, . . . 100 parts.

The (*c*) solution answers best for most purposes, though either of the above does well. The more dilute the stain, and the longer the staining process, the better the results.

Cedar oil, clove oil, bergamot oil, and xylol.

Turpentine.

Glycerine.

Dammar varnish.

Canada balsam dissolved in turpentine, benzole, or xylol.

Summary of Process of Examination of Fluids or Tissues for Micro-organisms.

37. Examine in a fresh condition fluid scraped from an organ or taken from a cultivation (§ 31, p. 45). Treat with various reagents (§ 21, p. 24). Dry and coagulate by heat and stain (§ 22, p. 26), or stain by His's method, if there is no albumen in the fluid (§ 32, p. 46).

Stain with methyl blue (watery solution) or some other aniline staining fluid, and mount at once in glycerine.

It is often a good plan to overstain, and then, before mounting, to wash out the excess of stain with a dilute solution (ten per cent.) of acetic acid.

After staining, wash off excess of fluid or of acid with distilled water (Fig. 13). Examine the specimen at once, or dry and mount in balsam.

Examine fresh sections or pieces teased out, both unstained (§ 21, p. 24) and stained (§ 24, p. 31, and § 25, p. 35); use various reagents (§ 21, p. 25).

Harden pieces of the tissue (§ 33, p. 47), and cut sections.

Soak in absolute alcohol.

Stain with gentian violet (§ 25, p. 35).

Wash in iodine and iodide of potassium solution (§ 25, p. 35).

Then wash in alcohol.

Transfer to alcoholic eosin solution (saturated).

FIG. 13.—Diagram to show method of washing off extra stain.
a. Wash bottle.
b. Cover-glass held in platinum-pointed spring forceps.

Clear up in bergamot or cedar oil.

Mount in xylol balsam.

Other methods, to which reference has been made, may also be used, but the above gives the most constant and satisfactory results.

For special bacteria, such as tubercle bacilli, typhoid bacilli, and others, special methods of treatment must be adopted (§ 26, p. 37 *et seq.*).

N.B.—Make a drawing of all micro-organisms about which your knowledge is not accurate, as they may have faded from view when next you come to examine them.

CHAPTER III.

SOLID CULTIVATION MEDIA.

Nutrient Soils.

38. To cultivate any organism artificially, apart from the tissue of its host, it must be supplied with a nutrient soil, which will support its vital necessities, and which is perfectly free from the presence of any other organism. If the soil be lacking in the former respect, the organism rapidly dies from inanition; if in the latter, a struggle for existence occurs between the organism already present and that newly implanted, and one or other is shortly exterminated, or each is modified in many respects from the close contact of so near a neighbour. Perfect sterility of the soil is therefore a *sine qua non* for the successful artificial culture of organisms.

The nutrient soil used may be either a liquid or a solid; and certain facilities of observation are offered by one or the other in the case of different organisms. The employment of solid media is now generally adopted, for in them an organism frequently exhibits specifically characteristic methods of growth in a way which is not attainable where liquids are used. On the other hand, speaking generally, organisms grow with greater rapidity in liquids than in solids, and thus sooner reach their state of maturity, and the transparency of most liquids so used affords facility in making observations, which is not the case with many of the solid materials. A few solid media, however, have been devised, in which the great advantage of transparency has been attained, and these offer such a series of advantages over other methods that they are now very largely employed in making observations. Their advantages are as follows:—

1. Convenience in manipulation: can be inverted (see footnote, p. 90, on gravitation of germs).
2. Slower growth of organisms, allowing more complete observation of the various stages of development.

3. Specific peculiarities in the mode of growth are more marked than in the liquid media. In some solids (*e.g.*, gelatine) the naked eye appearances are in many cases absolutely diagnostic of the presence of different species.

In the present chapter, the preparation of solid media, such as are most frequently employed, will be considered, and each step in the process will be described *seriatim*. The nutrient qualities of the soil differ in the case of each solid used, some materials being specially suited to the maintenance of one form of organism, others of another. Sterility of the soil is insured by the employment of heat, the most powerful sterilising agent at command. It may be attained either by the application of a very great heat for a short time, or by prolonged exposure to a much lower temperature. The action of steam at 100° C. is specially powerful in this direction; a comparatively short period of exposure to this being sufficient to effect complete sterility. As compared with dry heat at the same temperature, it is greatly more potent, probably from the fact that in the hot air the germs merely become perfectly dry, in which state they may be able to resist a very high temperature.[1] When moistened by the aqueous vapour, they are much more susceptible to the germicidal action of heat, which in all probability produces coagulation of some element essential to their life. The heat thus employed must be adapted in its character to the nature of the material to be sterilised, substances that might become too dry being steamed; others, where partial evaporation is desirable, being sterilised in a hot air chamber.

A particular account of the modes of preparation follows, accompanied in each case by the methods of inoculation for artificial culture, and the chief uses which each method of culture subserves.

(*A.*) Cultivation on Sterilised Potatoes.

39. For this purpose medium-sized potatoes, oval in shape and regular in contour, are selected. After a thorough washing in water, any excrescences are removed, and a preliminary purification is effected

[1] According to Pasteur, the spores of *Pencillium* and other common mucedines are not destroyed by a temperature of 120 to 125° C. *in the absence of moisture* (see "Bacteria," Magnin and Sternberg, p. 171).

by soaking them in a half per cent. solution of perchloride of mercury for three quarters of an hour; they are then rinsed in distilled water and are submitted to the action of the "steam steriliser," as devised by Koch (Fig. 14). This consists of a cylindrical tin receiver, provided with a conical lid, and covered completely with a closely fitting jacket of felt, to avoid too rapid radiation of heat. Internally, at the junction of the lower and middle thirds of the receiver, is a perforated diaphragm, which separates the lower or water chamber from the upper or steam chamber. The water chamber is furnished with a strong floor to withstand the heat of a powerful gas burner applied beneath it; and the steam thus generated in the lower chamber passes through the perforated diaphragm, and fills the steam chamber above. The water chamber is provided with a side register tube to indicate the depth of water present in it, which should never be less than three or four inches. The conical lid of the steriliser is perforated at its apex to admit the stem and bulb of a thermometer, which registers the temperature to which the contents of the steam chamber are subjected.

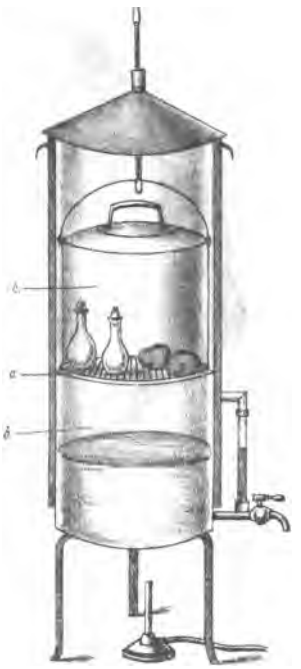

FIG. 14.—"Steam steriliser."
a. Diaphragm.
b. Water chamber.
c. Steam chamber, in which is potato receiver with perforated floor.

Into the upper chamber can be lowered the potato-holder, a cylindrical tin receptacle, with a perforated floor, which fits loosely within the steam steriliser, and, resting on the perforated diaphragm, allows its contents to be exposed to the steam as it rises from the lower to the upper chamber. The potato-holder is charged with the requisite number of previously purified potatoes, and is placed in the steriliser when the contained steam has reached close upon 100° C. The potatoes are left for thirty minutes (a few minutes more or less

respectively, if the potatoes are of very large or small size) in this position, and the water must be kept boiling the whole of that time.

FIG. 15.—*Bacillus anthracis* growing on sterilised potato. The growth has a succulent appearance, and forms an elevated plateau with rounded edges.

The potato-holder is now removed, and is placed on a layer of filter paper, soaked with a one per cent. solution of perchloride of mercury, to prevent contamination from the ingress of germs from below carried by currents of heated air, whilst the lid is very slightly raised at one side to allow the steam to escape, and at the same time to prevent access of germs from above.

Whilst they are thus cooling, preparations must be made for receiving the potatoes into sterile chambers. Shallow glass bells (Fig. 17) are prepared for their reception, by thoroughly rinsing them with the corrosive sublimate solution ($HgCl_2$ 1°/$_o$). The lower half of each bell is provided with a double layer of filter paper, which accurately fits its floor, and this is also soaked with the same solution. The bell thus prepared is allowed to stand an hour or two before use, to permit of subsidence of dust and spores in the contained air, and is kept carefully closed till the moment when the sterile potatoes are introduced.

In the meantime, also, a series of knives, equal in number to the potatoes to be used, must be prepared to divide the sterile potatoes,

FIG. 16.—*Pink Torula* growing on sterilised potato. The rose-coloured pigment is contained within the cells of the growth, each of which possesses a delicate yellow tint under the microscope. The faint white film on the surface of the growth is due to an accidental concomitant (a small *bacillus*).

without at the same time in any way contaminating the cut surface. For this purpose, ordinary kitchen knives are employed, without any

FIG. 17.—Glass dish and bell for sterilised potatoes.

rim at the back of the blade, which can thus slip through the steamed potato mass with but slight disturbance to it. A separate knife is to

be used for each potato, and, immediately before use the blade must be heated red hot, and then placed to cool, with its edge upwards,

FIG. 18.—*Micrococcus of acute osteo-myelitis* growing on sterilised potato. The yellow pigment consists of irregular granules, which lie around and not within the individual cells of the growth.

projecting over the side of a table, so as to offer as small a surface as possible to the gravitating atmospheric germs.

The potatoes being now cool enough for manipulation, the operator cleanses his hands thoroughly with corrosive solution (1-1000), and, getting an assistant to raise the lid slightly for a moment in a vertical direction, removes one potato from the holder. The potato is grasped in its shortest diameter (see Fig. 20), and, thus held, it is cut through in its longest diameter by a single steady sweep of one of the sterilised knives. The assistant now raises the lid of one of the sterile bells, slowly and perpendicularly, and when the knife has all but completely divided the potato, the latter is laid on its side on the floor of the chamber, and its upper cut half is opened up, hinge-like, by the blade of the knife, and laid alongside the lower half of the potato with its large smooth-cut surface upwards. The cut sur-

faces of the potato have thus only come in contact with the blade of a knife immediately previously sterilised, and the risks of contamina-

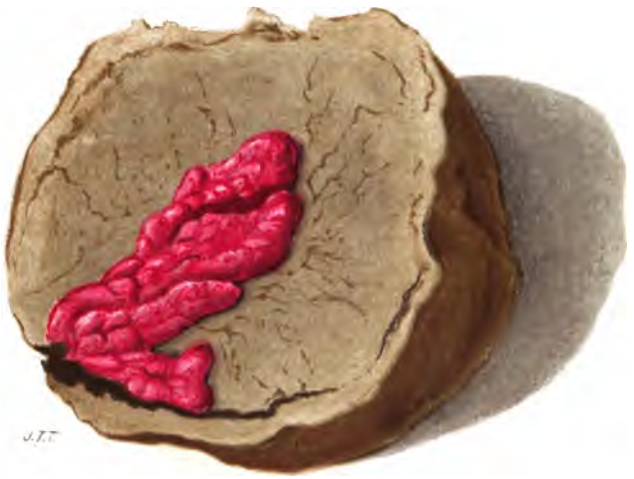

FIG. 19.—Growth of *Micrococcus prodigiosus* on sterilised potato. The brilliant growth stands out very sharply from the slightly discoloured and cracked potato.

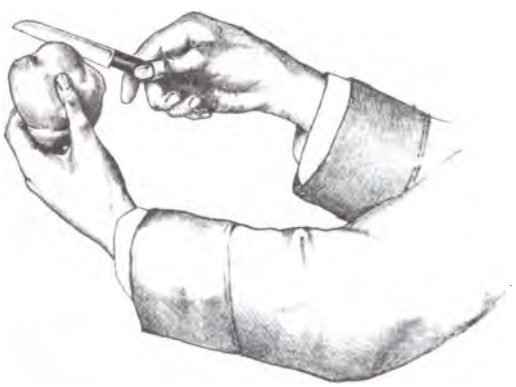

FIG. 20.—Method of dividing sterilised potatoes.

tion are minimised. The knife is now rapidly withdrawn, and the bell jar replaced.

Summary of Process for preparing Sterile Potatoes.

Cleanse potatoes.

Steep in one-half per cent. solution of corrosive sublimate for forty-five minutes.

Rinse with distilled water.

Steam for thirty minutes at 100° C.

Divide with knives which have been previously heated red hot.

Place in sterile bell jars, purified with one per cent. solution of corrosive sublimate.

Inoculation of Potatoes.

40. Such potatoes should either be inoculated at once with seed material, or allowed to stand under observation for four days before this is done. In the former case the implanted organisms have the start of any which may have gained access during the processes of preparation or of inoculation. In the latter, if no reaction take place, one may be quite sure that the soil to be inoculated is perfectly sterile. In inoculating sterile potatoes, as also the various other soils, for the growth of organisms subsequently to be described, a platinum wire mounted upon a glass rod (see Fig. 21) is used. This is sterilised immediately before use by heating it white hot in the flame of a Bunsen burner. The upper part of the glass rod is also heated. In a few seconds the wire is cool enough to be used without damaging the seed material, which it is to transfer to the sterile potato surface. The wire, sterilised and charged with a small quantity of the seed material, is held in one hand; with the other the operator slowly raises the upper glass bell at one side, so as just to allow of the entrance of the wire. The end of the wire is now rubbed gently upon the centre

Fig. 21.—Platinum wires used for making inoculations.
a. Straight wire for inoculating gelatine, bread paste, &c.
b. Looped wire for liquids.
c. Hooked wire for inoculating solidified blood serum and potatoes.
d. Bottle, with wadding on which glass rods rest.

of the cut potato surface, and the seed material is thus implanted in the sterile nutrient soil (see Fig. 22). The wire is then withdrawn, and the

FIG. 22.—Inoculation of potatoes.

bell jar closed. Note is taken of the date and the substance inoculated, and the potatoes are set aside for subsequent observation.

As a general rule, those organisms which are adapted for cultivation on potato are capable of growing actively at ordinary temperatures. In any special case, where it is desired to incubate organisms growing on this soil, the bell jar, with its contents, must be placed in a warmer room, or in one of the larger sizes of incubator subsequently to be described. Incubation at high temperature is not so desirable in the case of potatoes as in that of some other soils, for they become dry superficially, and thus lose their special adaptability to the growth of organisms.

(*B.*) CULTIVATION ON STERILE BREAD PASTE.

41. Slight differences in the chemical constitution of a nutrient soil often determine its suitability, or otherwise, for the growth of an organism; certain species, which thrive but slightly on potato, are found to flourish luxuriantly when supplied with other forms of starch, as that from cereals. A convenient soil for such organisms is supplied by bread paste. This is made simply of bread crumb and water. A stale loaf is thoroughly dried in an oven; then all the crust is removed, and the remainder is finely powdered. Small clean one-eighth litre Bohemian flasks are charged each with ten grammes of the bread crumb, and to this is added 2·5 c.c. of dis-

tilled water. The flasks are provided with compact cotton wool plugs. To make these, a square piece is taken from a sheet of

FIG. 23.—*Mucor Lichtheimii* growing on bread paste.
On the surface of the delicate growth, small translucent yellow beads of liquid (whichs give an alkaline reaction) are seen.

cotton wool, and the corners consecutively turned inwards towards the centre, till a firm round mass is produced. This is pushed into

the neck of the flask, with its smooth compact end downwards, which adapts itself closely to the smooth inner surface of the neck of the

FIG. 24.—*Aspergillus albus* growing on bread paste.

flask. When such a flask has been sterilised, the firm plug acts as a mechanical filter, allowing free interchange of air between the atmo-

sphere inside and that outside the flask, but preventing all ingress of germs from without. The preparation of these plugs is a matter of

FIG. 25.—*Aspergillus repens* growing on bread paste—commencing growth.

great importance, for upon their effectiveness as filters depends the purity of artificial cultures, not only in this special mode of cultivation, but in several of those to be mentioned subsequently.

The steam steriliser is now prepared, and when the water is boiling the flasks are placed in the steam chamber, where they remain for

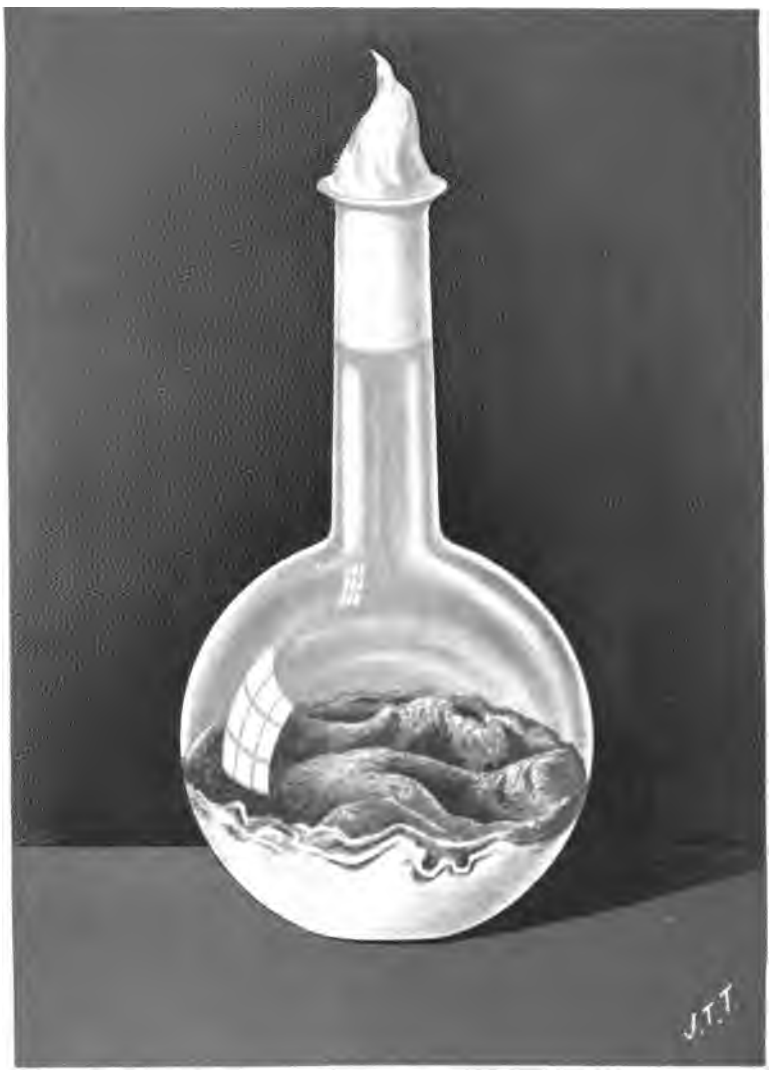

FIG. 26.—*Aspergillus repens* growing on bread paste (extension of surface of growth causing undulations.)

half an hour. They are then removed, and placed in an ice-press for twenty-four hours, and then once more steamed for thirty minutes.

Their sterility is now attained; but to obviate any risk of its being incomplete, it is well to keep them under observation for a week before using them; when, if no reaction has occurred, they may safely be employed as sterile soil.

Inoculation of Bread Paste.

42. The platinum wire already described is used for the inoculation. A flask containing sterile bread paste is inverted, and held thus in the left hand (see Fig. 27). Its cotton wool plug is then twisted round, to break down any adhesions between it and

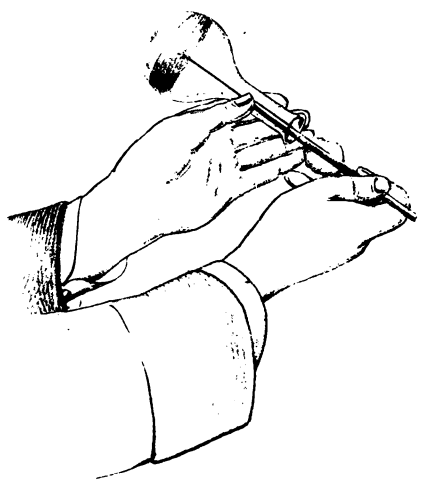

Fig. 27.—Inoculation of bread paste.

the neck of the flask, and is then withdrawn, and held between two fingers of the same hand, with its lower end pointing towards the ground. This arrangement is to avoid risks from the gravitation of germs through the atmosphere. The platinum wire is now sterilised, charged with seed material, and its tip repeatedly plunged into the mass of paste at the bottom of the sterile flask. It is then withdrawn,

the flask plugged, and only then is it restored to its upright position. During all these manipulations the operator is careful not to breathe

FIG. 28.—*Aspergillus nigrescens* growing on bread paste.

upon the flask, plug, or inoculating wire, and his hands are previously washed in a 1 per 1000 solution of perchloride of mercury, to destroy

germs which might be carried upwards into the flask with the hot air currents which are constantly rising from all the free surfaces of the

FIG. 29.—Micrococcus of osteo-myelitis growing on bread paste.

body.[1] The inoculated bread paste may with advantage be incubated at summer or at blood heat, in any of the forms of incubator em-

[1] "Essays on the Floating Matter of the Air in relation to Putrefaction and Infection"—Tyndall: London, 1881.

ployed. Most organisms will also grow upon this soil at ordinary temperatures, but more slowly than when incubated. By slightly

FIG. 30.—Black *Torula* growing on bread paste.

varying the proportion of water in the process of preparation, a more or less succulent paste can be produced, and the requirements of a

variety of organisms thus met. This form of soil is specially adapted for the growth of the various species of moulds—*Aspergilli*, *Mucores*, and *Penicillia*.

Summary of Process for preparing Sterile Bread Paste.

First Day.—Place ten grammes bread crumb and 2·5 c.c. water in a glass flask. Plug with cotton wool.
Steam flask at 100° C. for thirty minutes.
Second Day.—Repeat steaming for thirty minutes.

(*C.*) Cultivation in Sterile Nutrient Jelly.

48. In the nutrient soils already described we have had to deal with an opaque substratum, on which only the surface appearances of the cultivated organism can be distinguished. In order that the more intimate relations and tendencies of the growth may be observed, it is desirable that some material should be devised which combines sufficiently nourishing qualities with solidity and transparency. These conditions are realised by the peptonised meat jelly devised by Koch, in which a highly concentrated meat infusion is rendered solid by the admixture of gelatine, its nutrient qualities enhanced by the addition of peptones, and its perfect transparency ensured by careful filtration, or, if need be, by clarification. The method of its preparation is as follows:—To two pounds of finely minced lean meat (pork or beef by preference) are added two litres of distilled water. These are well stirred together, and allowed to stand for twenty-four hours in a cool place. The fat, which separates and floats on the surface, is then removed with a creaming ladle or clean filter paper, and the residue is poured into the cavity of a screw press (Fig. 31), lined with a fine cotton cloth, through which the liquid portions are strained, and collected in a large glass flask. The mass of meat thus left is closely wrapped in the piece of cloth, and is subjected to repeated pressure in the screw press, so as to deprive it of all remaining liquid.

By this means a turbid red broth is obtained, which should measure two litres. If it measure less than this, add some distilled

water to the solid residue in the cloth, and express all moisture from the mass once more, adding sufficient of the liquid thus obtained

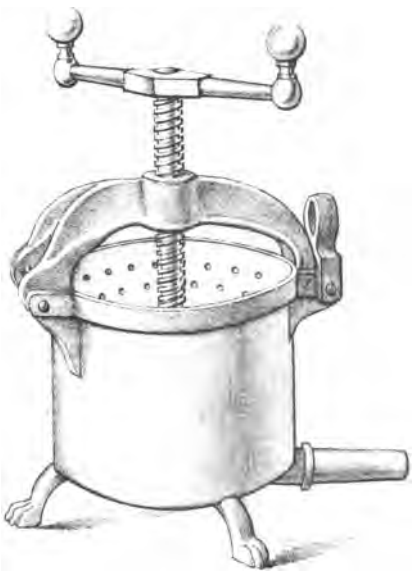

FIG. 31.—Screw press for extracting meat infusion for Koch's jelly.

to make up the bulk of the broth to two litres. Put the two litres of broth in a four-litre flask, and add to it 100 grammes (*i.e.*, five per cent.) of best table gelatine, 10 grammes (0·5 per cent.) of dry albumen peptone, and 5 grammes (0·25 per cent.) of common salt. The fine photographic gelatine now so much in use is not suitable for the purpose, as it does not give the requisite solidity, unless used in a much larger proportion. The gelatine must be of the best French variety (blue and gold label), prepared by "The Gelatine Manufacturing Company," Paris. The gelatine mixture is now stirred, and its reaction tested. This is nearly always found to be distinctly acid, which is not favourable to the growth of most organisms.[1] If the mixture be

[1] Certain genera (moulds) grow best in slightly acid solutions; others (*e.g.*, *Bacterium termo* and its congeners) in an alkaline solution; and others (chiefly the group of micrococci) in neutral solutions.

acid, it must be neutralised by the addition of a saturated solution of carbonate or alkaline phosphate of soda. This must be done very accurately, and is best accomplished by using pieces of red and blue litmus paper, adding the alkali in small quantities to the gelatine broth, until an exactly similar tint is produced in each test paper on its immersion in the liquid. As soon as its contents are completely neutral in reaction, the flask is stoppered with a plug of sterile cotton wool, and is placed in the steam steriliser already described (§ 39, p. 57), which has been previously raised to a temperature of 100° C. Here the flask and its contents are steamed for thirty minutes, after which they are allowed to stand for twenty-four hours in a cold place. On the third day of the preparatory process the flask is again steamed for thirty minutes, and while still liquid the contents are once more tested, and more of the soda solution added, if they prove acid. If already slightly alkaline, the broth may be left without further treatment, as slight alkalinity of the soil is not unfavourable to the growth of many species of micro-organisms. Whilst still liquid, the peptonised broth should be filtered through coarse filter paper in a heated funnel

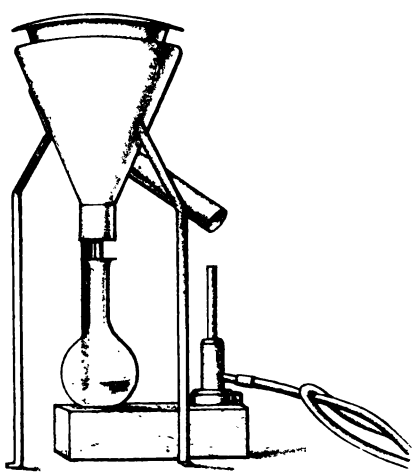

FIG. 32.—Hot water funnel for filtering meat jelly.

(see Fig. 32). This consists of a glass funnel, the neck of which fits into an indiarubber plug, and around which is a copper funnel of

larger dimensions, fitting as a collar, and leaving an interspace between the two funnels, which acts as a water chamber. Hot water is placed in this chamber, and its heat maintained by the application of a Bunsen burner to a copper diverticulum connected with its cavity. The filter paper is washed with boiled distilled water, to remove any fine separable particles from it; and the gelatine solution first passed through it is reserved for a second filtering, so as to secure a perfectly pellucid filtrate. During the process of filtration the funnel is kept covered with a glass plate or bell, to prevent ingress of dust from the air. The filtrate is received into sterile glass flasks, stoppered with sterile cotton wool plugs. These are prepared as follows:—The flasks (one quarter litre) are thoroughly washed and dried, and then plugged with cotton wool. The stoppered empty flasks are then submitted to a dry heat of 170° C. for an hour, which effects their complete sterility. The plug is then removed from the mouth of one of these sterile flasks, and held with a pair of catch forceps, so that it touches no other object, its lower aspect at the same time being directed downwards, so as to avoid deposition upon it of the dust particles settling from the air. The flask is placed below the filter, and the gelatine is allowed to pass into it till it is about one-quarter full. It is then quickly plugged, and another flask introduced in its place. A flask so charged with gelatine broth is called a stock-flask. The flasks, thus charged with the clear filtrate, and at once plugged with cotton, are then placed in the steriliser and steamed for twenty minutes. If they are as much as half full, there is danger of the contents rising as high as the cotton wool plug, whence during the steaming process a quantity of particles may be washed down, which interfere with the perfect transparency of the nutrient jelly. If the filtrate is not perfectly clear, it must either be refiltered, or it must be clarified with egg albumen. This is effected as follows:—The white and shell of an egg are beaten up into a frothy mass, and added to the whole supply of turbid gelatine broth. This is then placed in the steam steriliser, and steamed until coagulation of the egg albumen takes place. By the action of heat all the egg albumen is precipitated, and it carries down with it the fine particles suspended in the broth, and leaves, when separated by filtration, a perfectly clear nutrient substance. At the end of the third day's steaming, the gelatine broth, now in stock-

flasks, is placed once more in a cold place for twenty-four hours. On the fourth and fifth days the jelly is again steamed for a quarter of an hour each day, standing in the intervals of heating in a cold place. The process of sterilisation is now complete. This process is based on Tyndall's principle of sterilising by the employment of discontinuous heat; only here the partial loss of watery elements which necessarily accompanies ebullition is avoided by the use of the steam steriliser, which prevents all evaporation, and is equally effective in producing sterility. Klein recommends that a glass cap should be used to prevent the deposition of dust on the cotton wadding plug.

Summary of Process for preparing Sterile Nutrient Jelly.

First Day.—Steep two pounds of meat in two litres of water for twenty-four hours.

Second Day.—Extract, and filter broth. It should measure two litres.

„ Add 100 grammes gelatine, 10 grammes albumen peptone, and 5 grammes common salt.

„ Neutralise with carbonate of soda (sat. sol.).

„ Steam at 100° C. for thirty minutes.

Third Day.—Steam thirty minutes; filter through hot water filter into stock-flasks; steam in stock-flasks twenty minutes.

Fourth Day.—Steam stock-flasks fifteen minutes.

Fifth Day.—Steam stock-flasks fifteen minutes.

In addition to the formula given above for preparing a nutrient jelly, the following will be found useful (modified from Klein):—

Distilled water, ...	100 parts.
Beef peptones (Savory and Moore), . .	2 parts.
Cane sugar, ...	1 part.
Gelatine, ...	10 parts.[1]

Dissolve, neutralise with carbonate of soda solution, and then sterilise by discontinuous steaming as above.

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[1] Owing to the presence of sugar in this formula, a large percentage of gelatine is required to render the material solid at ordinary temperatures.

Another valuable formula is as follows:—

Darby's fluid meat (peptonised),	1 part.
Distilled water,	100 parts.
Gelatine,	5 parts.

Neutralise, and sterilise as above.

Methods of employing Sterile Nutrient Jelly.

(*A.*) *In Test-Tubes.*

44. The test-tubes are first prepared by thorough cleansing, and then plugged with firm cotton wool plugs, and heated for an hour to 170° C.

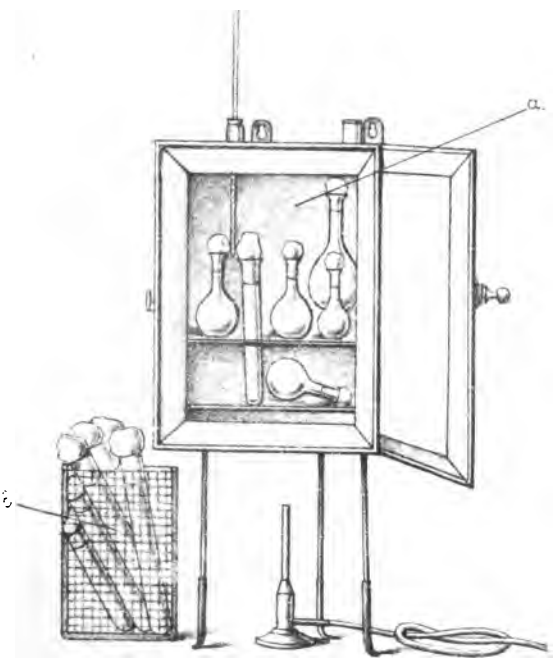

Fig. 33.—Hot air chamber for sterilising glass, wadding, and other apparatus.

a. Hot air chamber containing test-tubes and flasks.

b. Wire basket containing test-tubes to be sterilised.

When hung upon a wall, a sheet of asbestos should intervene between the apparatus and the wall.

in the dry air steriliser (see Fig. 33). When cool, they are ready

to be charged with the nutrient jelly. The contents of one of the sterile stock-flasks are liquefied by heating, and a glass pipette is sterilised preparatory to transferring some of the stock jelly to the test-tubes. The pipette is either superheated in the flame of a Bunsen burner, or exposed to a temperature of 170° C. in the dry air steriliser for an hour, or it may be thoroughly washed, first with a mercuric perchloride solution (aqueous 1 in 1000), and then in absolute alcohol, and dried. The sterile pipette is introduced into one of the stock-flasks, and 10 c.c. of the liquefied contents drawn up into it by suction. The upper end of the pipette is then stoppered by the finger to prevent its contents from escaping, until they are introduced into one of the sterile test-tubes. The sterile plug is removed from one of these, and the liquefied jelly is allowed to flow slowly to the bottom of the tube, care being taken that the liquid do not touch the sides of the tube in its descent. The cotton wool plug is then returned, and the tube, with its contents, is placed upright, and allowed to cool. The 10 c.c. of jelly which the tube contains soon solidifies, and the tubes thus prepared, if they show no reaction after the lapse of a week, may be assumed to be sterile, and used as a cultivating soil for micro-organisms. If the atmosphere is densely germ-laden (as during the summer and autumn months), the transference of jelly from the stock-flasks to test-tubes should be effected under the carbolic spray,[1] or the test-tubes, when

[1] The question as to whether the carbolic spray is a reliable means of preventing contamination in cultivations in those manipulations which necessitate a free access of the atmosphere to the sterile media, is one of great importance. During certain periods of the year the atmosphere is so densely germ-laden as to render it a matter of the greatest difficulty to prevent septic contamination. Our experience proves that if an efficient spray be employed, this danger can be entirely avoided. The spray used must produce a vapour in which carbolic acid is present at least in the proportion of one part to thirty parts of water. A spray cloud of this strength is an efficient antiseptic, and, applied under these conditions, the steam spray is a weapon of precision that may be thoroughly depended on. And in judging of the efficiency of an antiseptic for these purposes, a sufficient standard of comparison is supplied by the ordinary spores usually present in the atmosphere; for if an antiseptic be powerful enough to destroy these, it should be considered efficient, and its potency should not be judged by its ability or inability to destroy certain rare and very resistant living structures, such as the spores of *Bacillus anthracis*. A comparison based on the latter data is to some extent misleading, and has tended to throw discredit on some antiseptics which general experience has proved to be useful and reliable.

charged, should be once more submitted to ten minutes steaming on each of two successive days, by which is avoided the danger of contamination by the atmosphere, during the manipulative process.

The stock of test-tubes thus prepared is kept under observation for seven or eight days before it is used as a cultivating medium. If any organisms have gained access to the jelly in the process of preparation, they will become apparent during this period by altering its appearance. In this way can be excluded any danger of the development of foreign elements alongside the special cultures established in the test-tubes, which would render useless any results obtained. The method of inoculating the nutrient jelly is as follows:—A short piece of platinum wire is mounted on a solid glass rod (see Fig. 21), and is sterilised by heating to a white heat in the flame of a Bunsen burner. It is then allowed to cool, without touching any free surface, whence it might derive contaminating matter. After it is loaded with a minute trace of the seed material, the organisms contained in which it is desired to cultivate, it is introduced into the sterile tube. The test-tube is held inverted in the left hand (§ 42, p. 68), and the plug of cotton wool is twisted once or twice in the mouth of the test-tube, to break down any adhesions between it and the neck of the tube which might prevent its rapid removal.[1] The plug is then removed from the mouth of the test-tube, and held between two of the unoccupied fingers of the left hand (see Fig. 34), care being taken that no part of it which passes within the test-tube comes in contact with any source of infection other than the air itself; at the same time this portion of the plug is directed downwards in order to avoid the germs gravitating through the atmosphere. The platinum wire is now plunged twice or thrice into the gelatine mass, and gently removed, so that two or three linear tracts are produced in which the particles of the inoculated material are deposited in a widely dispersed condition; and along the linear tracts thus produced, appearances may be subsequently observed, which in many cases are characteristic of the special kind of seed material which has been sown. The platinum

[1] If the plugs are dusty, it is well to burn the outer surface of the plug by passing it rapidly through a flame before removing it from the test-tube. The wadding burns very rapidly, and must be extinguished at once.

wire is now withdrawn, and the cotton wool plug is firmly replaced in the mouth of the test-tube. All these manipulations must be carried

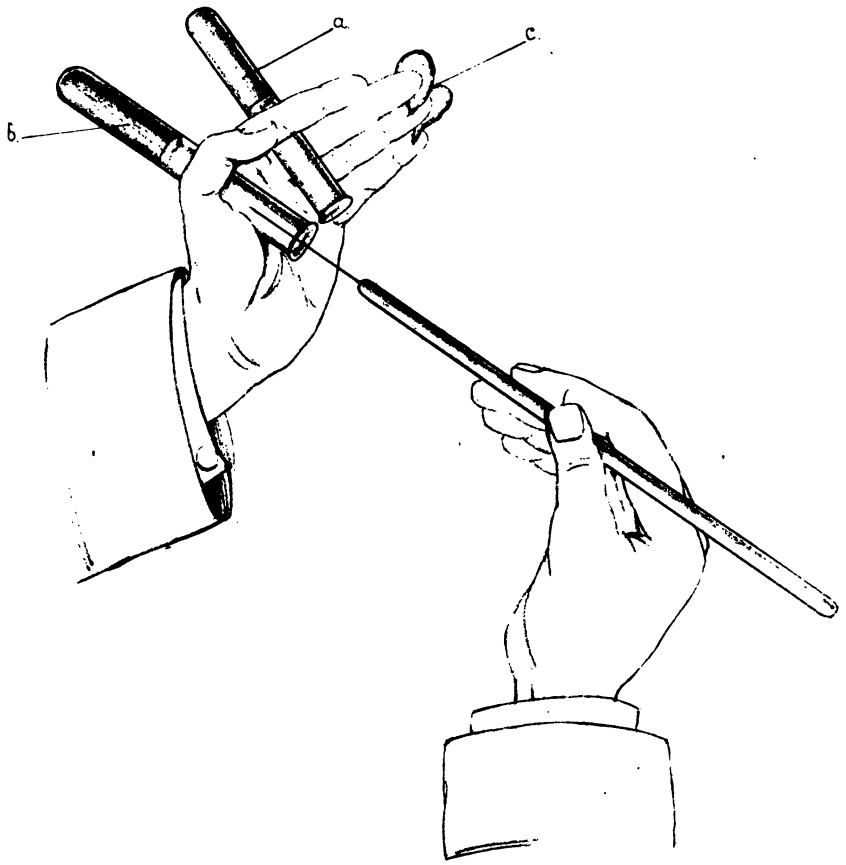

FIG. 34.—Method of inoculation in test-tubes containing nutrient jelly.

a. Cultivation in jelly, from which inoculation is to be made.
b. Tube containing sterile jelly, into which the straight needle is introduced.
c. Cotton wadding plugs held between the tips of the fingers. (The hand is held in such a position that these plugs have their smooth surfaces downwards.)

out in a perfectly still atmosphere, or under the protection of the carbolic spray. The latter method specially offers many advantages:

organisms inoculated in the antiseptic vapour do not thereby lose their vitality, while, if the spray be an efficient one, all

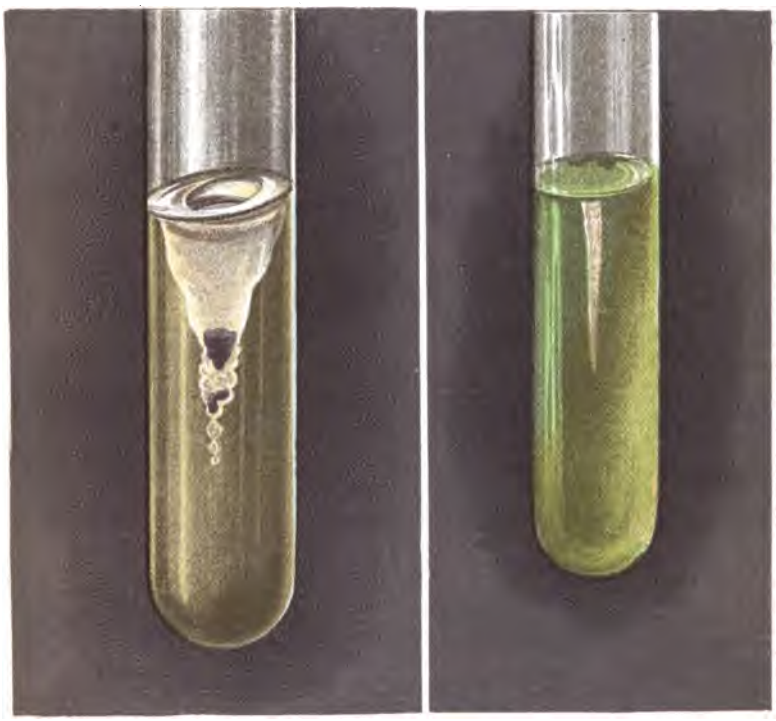

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FIG. 35. FIG. 36.

FIG. 35.—Growth of violet bacillus in nutrient jelly. The growth commences first near the surface, along the track of the needle. Liquefaction rapidly takes place, the liquefied material forming a kind of inverted cone, the apex of which is made up of small beads or globes, in which the violet masses of bacillus are deposited.

There is a violet scum floating on the surface of the slightly turbid liquid ; if the test-tube be shaken slightly, part of this sinks. Near the apex of the cone, and on the floor of the small beads, masses of the violet bacillus are seen.

FIG. 36.—Fluorescing bacillus growing in nutrient jelly. A delicate green, slightly opalescent, film is seen on the surface of the jelly. A pale grey growth is seen along the track of the needle. The surrounding jelly gradually assumes a peculiar green fluorescent appearance, which alters very considerably according to the background and the lighting used. This resembles the bacillus of blue milk in many respects, especially below the surface. The surface growths, however, differ very materially.

danger of contamination from the atmosphere is with certainty excluded.

Nutrient jelly in test-tubes is specially adapted for the cultivation of those organisms which can subsist on concentrated animal food material, and which grow at ordinary temperatures. At blood-heat the five per cent. jelly becomes liquid, and thus it is not adapted for the cultivation of organisms, which require so high a temperature to maintain their growth. Notwithstanding these limitations to its use,

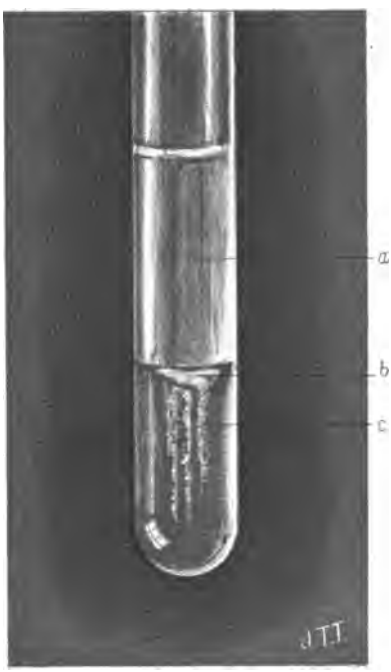

FIG. 37.—Cultivation of *B. anthracis* in nutrient jelly, showing a liquefaction of the upper portion.
b. Dull yellow mass, composed of accumulated bacilli.
c. Bacilli growing along the tracks of the inoculating needle.

it is found to suit the requirements of a great number of species, and is the more useful from its giving a characteristic reaction in the presence of several of them. Organisms vary very much in the reaction which they occasion in the jelly; some have the peculiarity

of liquefying it; others produce nodules, delicate or thick cloud-masses, spicular or branched growths; others have a distinct colour reaction, by which they may at once be recognised (Figs. 35 and 36), and the same holds true with regard to odours characteristic of certain species. But this last feature avails but little in differentiating species

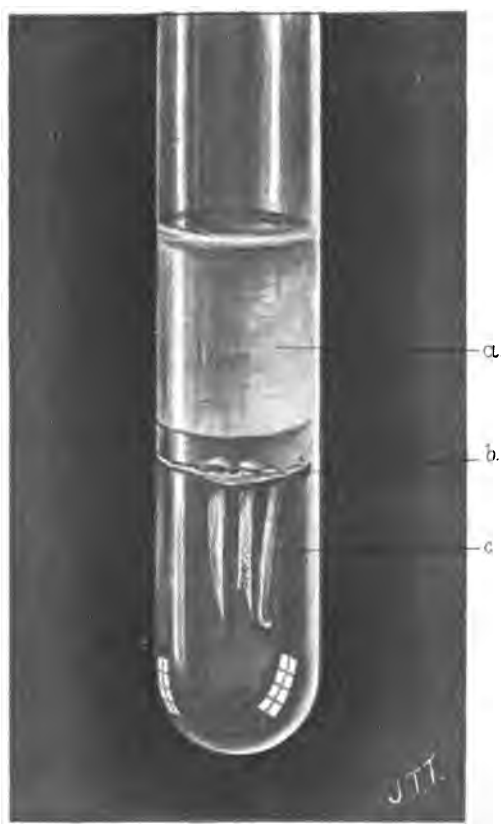

FIG. 38.—Cultivation in nutrient jelly of micrococcus found in osteo-myelitis.

a. Liquefied upper layer.

b. Sediment from liquefied portion, forming an orange-yellow mass of micrococci.

c. Growths of the micrococcus along the tracks of the needle.

generally, as a classification of odours on a scientific basis is still a desideratum in many branches of biological research.

One of the best known species cultivated in gelatine is the *Bacillus anthracis* (Figs. 37 and 43), which grows downwards in the track of the inoculating wire in small round nodules of the size of grains of sand, and of a dull pale yellow colour, and gradually liquefies the contents

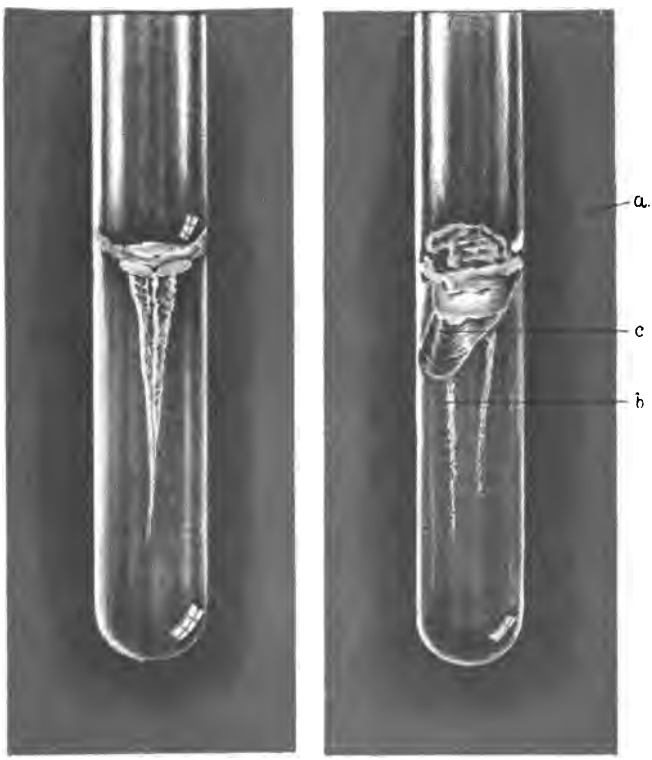

FIG. 39. FIG. 40.

FIG. 39.—Cultivation of pink yeast in nutrient jelly, showing the brighter pink colour of the surface growth.

FIG. 40.—Cultivation of micrococci found in vaccine lymph.
a. Yellow growth on the surface.
b. Growth of small rounded white masses along the tracks of the needle.
c. Cleft in the gelatine, from increased surface tension produced by evaporation.

of the test-tube from above downwards, till in the course of six to eight weeks the whole mass has become liquid. At the bottom of the

liquid is a dense amorphous precipitate of a dull yellow colour, passing in older specimens to an orange yellow hue, which consists of anthrax bacilli, produced by the constant longitudinal growth and transverse cleavage of the anthrax rods which were in the first place implanted.[1]

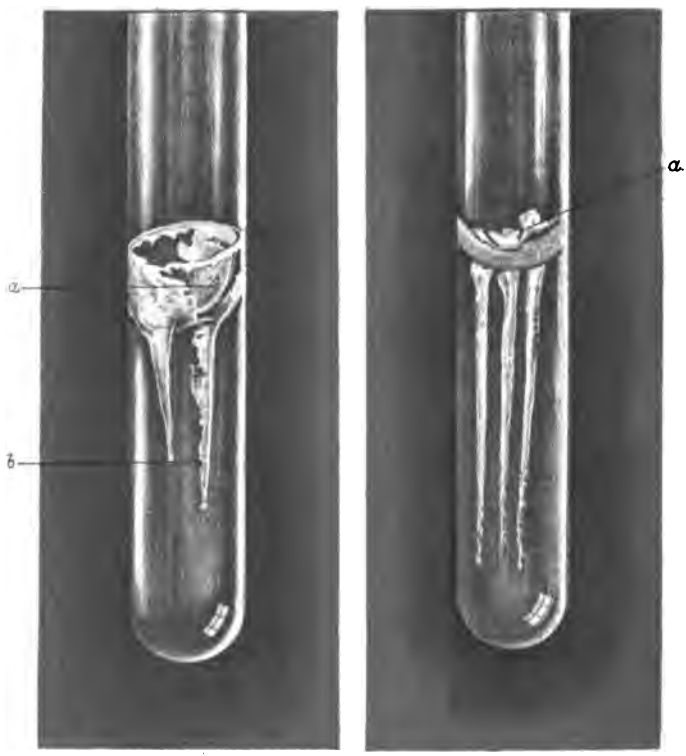

FIG. 41. FIG. 42.

FIG. 41.—Organism of the rabbit septicæmia (bacillus).
a. Well-marked surface growth.
Hollowing of surface of gelatine due to evaporation.
b. Growth along track of needle, also well marked.

FIG. 42.—Cultivation of micrococci found in vaccine lymph.
a. White growth on the surface. (Compare Fig. 40.)

Another organism which develops in a distinctive manner in peptonised nutrient jelly is the pink yeast plant. It grows most luxuri-

[1] Cp. Klein: Rep. to Med. Off. Local Government Board, 1882.

antly on the surface of the jelly, where it is found heaped up around the point of inoculation, with a slightly elevated free surface of a bright rose pink colour. It also grows downwards into the jelly, following the line of inoculation, and presenting the appearance of a smooth sharp-pointed spike, of a dull grey pink colour. It does not cause liquefaction of the jelly, and after developing to the extent depicted in Fig. 39, it ceases to grow, and passes into a stage of quiescence; but it retains the power of further propagation, if transplanted, for an indefinite period.

Amongst the pathogenetic organisms which flourish in this medium is that which gives rise to acute osteo-myelitis (see Fig. 38), first fully described by Becker. It grows chiefly within the jelly, following the track of the inoculating wire, along which a series of very fine nodules is produced. These nodules have a faint yellow tinge, and are seen to be perfectly smooth in their outlines when looked at with a magnifying glass. On the sixth and seventh day after inoculation, liquefaction of the jelly commences, producing first a funnel-shaped depression full of liquefied jelly, and gradually extending, first to the periphery of the test-tube, and then downwards, till the whole of its contents become liquid. An amorphous deposit of a yellow colour is found at the bottom of the test-tube, and the whole of the superjacent liquid is slightly tinged with the same colour. Re-inoculations of culture material must be frequently repeated in the case of this organism, as its vitality is lost soon after it has exhausted all the available nourishment of one of the tubes of jelly, and it becomes quite inert unless it is speedily supplied with fresh pabulum.

When an observer is acquainted with the typical form of growth which a species exhibits in Koch's jelly, he has no difficulty in detecting the presence of impurities should they gain access to the contents of the tube. Modifications are at once apparent in the type of the growth which takes place, or an organism with quite distinctive characteristics may be seen growing alongside that already cultivated; an instance of this is supplied by Fig. 43, in which a cultivation of *Bacillus anthracis* is figured in which this accident has occurred. The upper part of the jelly has liquefied in the usual way, but at a lower part of the test-tube is an opaque spherical mass, seen with a pocket lens to consist of a central nodule, with delicate silky fibrils stretching

from it in all directions. This is at once recognised by its method of growth as a specimen of one of the cheese moulds (*Penicillium*

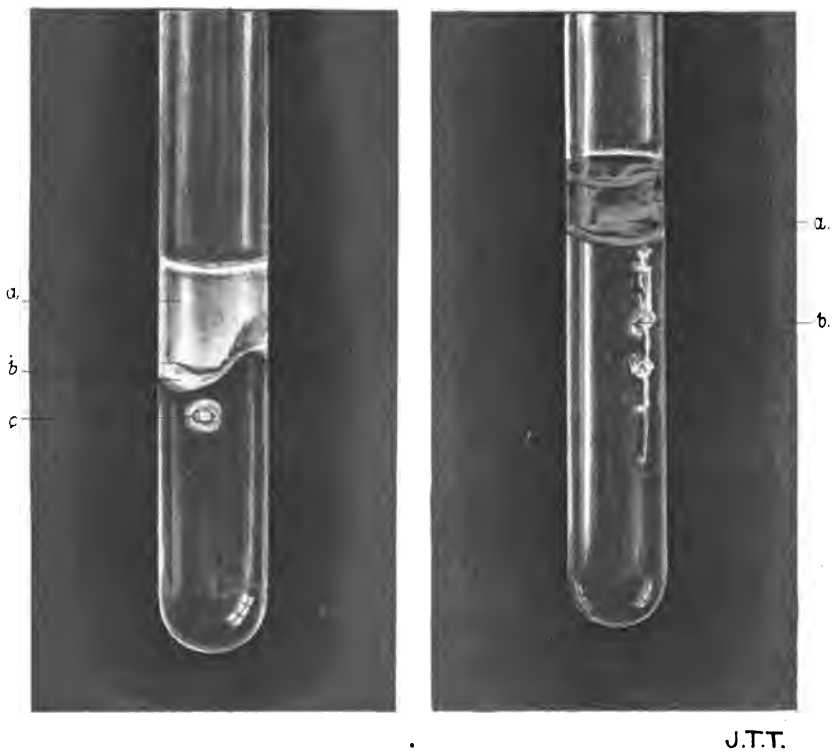

FIG. 43. FIG. 44.

FIG. 43.—Impure cultivation of *B. anthracis* in nutrient jelly.
a. Upper portion of jelly, liquefied.
b. Sediment of *B. anthracis.*
c. Nodule of *Penicillium glaucum.*

FIG. 44.—Growth of *Bacterium termo* and *Penicillia* in nutrient jelly.
a. Liquefied portion of jelly with opalescent green tinge, due to presence of *B. termo.*
b. Nodules of *Penicillium* growing in the track of the needle.
N.B.—The jelly was inoculated with a non-sterilised needle.

glaucum), the spores of which are almost universally present in the atmosphere; and the spherical nodule in the jelly represents the

outcome of the growth of a single spore, that has found its way into the jelly, in all probability by falling upon the inoculating wire whilst it was passing through the air to the sterile test-tube.

(*B.*) In Capsules, for testing Air.

45. The condition of the atmosphere, in respect to the number of germs in it, may be ascertained approximately by the employment of a cultivation experiment with sterile jelly. A glass jar plugged with cotton wool, and containing a shallow glass capsule (Fig. 45), is

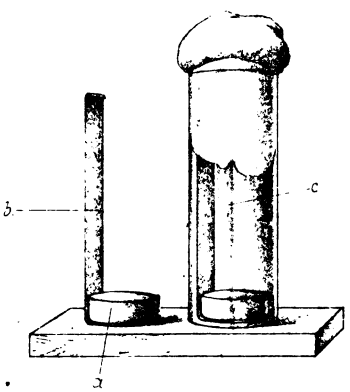

Fig. 45.—Apparatus for determining relative purity of atmospheres (qualitative).

a. Capsule containing sterilised nutrient jelly.
b. Brass lifter for removing capsule for examination.
c. Glass jar with sterilised plug, containing lifter and capsule ready for use.

sterilised by heating to 170° C. dry heat for one hour. When it has cooled, the glass capsule is charged with 10 c.c. of jelly, which has been previously liquefied by heat, and withdrawn from one of the stock-flasks by means of a sterilised (superheated) glass pipette. This has to be done with great care—firstly, to avoid the risk of contaminating the contents of the stock-flask; and, secondly, to prevent the introduction of dust particles along with the jelly into the sterile jar prepared for the experiment. The cotton wool plug of the stock-flask is half withdrawn, and the point of the pipette is slowly thrust through the remaining layers of the plug till it is free within

the cavity of the flask. Then the pipette is pushed down into the liquefied gelatine, 10 c.c. of which are drawn up into the bulb by suction. The upper end of the pipette is closed by the thumb, and the pipette withdrawn. During the withdrawal of the pipette the cotton wool plug is pressed firmly against it, to prevent ingress of dust particles; and so soon as the point of the pipette is disengaged from the cotton wool plug, that is replaced in its proper position.[1] The same manœuvres are carried out in introducing the pipette into the sterile jar; and when this is done, the liquid jelly is allowed to trickle down into the capsule, and the pipette removed with the same precautions as before. We have thus obtained a thin layer of a very susceptible substance, which may be exposed to any atmosphere; and the reaction that occurs can be easily observed through the walls of the glass jar without in any way disturbing the capsule in which it takes place. The jelly soon solidifies; and after being kept for a

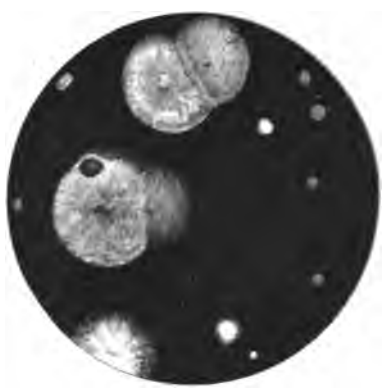

FIG. 46.—From a photograph of glass capsule exposed in Saint Giles' Cathedral, Edinburgh (see description).

week in a warm room, to make sure that no adventitious growth will take place in the capsule, it may be exposed to any given atmosphere by the removal of the cotton wool plug. This exposure is of a definite

[1] This method is open to the objection that it cannot be repeated safely more than once or twice, as the risks of contamination are greatly increased by the presence in the plug of even a small quantity of the nutrient material; a better plan is to remove the plugs from both beaker and flask, and carry out all the manipulations under the protection of the carbolic spray.

duration, and by comparing this with the number of foci of growth which appear subsequently in the jelly, one can calculate approximately the extent to which germs are present in the atmosphere that is tested. The result of such an experiment is shown in Fig. 46, which is taken from a photograph of a capsule exposed to the atmosphere of Saint Giles' Cathedral, Edinburgh, for a period of fifteen minutes. During that period sixteen germs fell into the capsule and produced the sixteen foci of growth, which could be counted in the capsule eight days after its exposure. The individual species of organisms present in an atmosphere may be recognised by the naked eye appearances of the resulting growths, or by putting the capsule upon the stage of the microscope, and examining its contents with a low power. In the case cited the organisms present were chiefly *Aspergilli*, *Penicillia*, and various *micrococci; Bacterium termo* did not appear, but a colony of pink *Torula* was at once recognised by its bright colour. In a subsequent series of experiments performed in the hot rooms of the Turkish Baths in Princes Street, Edinburgh, it was found that *Penicillium glaucum* greatly preponderated; *Bacterium termo* was also largely present, but the various forms of *micrococci* did not appear in the capsules.[1]

Another method, based on Cohn and Miflet's plan, by which the number of germs in any given bulk of atmosphere can be ascertained, is that of Hesse. His apparatus (Fig. 47) consists of a hollow glass cylinder, 18 inches in length and 2½ in diameter. over one of its ends is stretched a thin indiarubber sheeting, in the centre of which is a small pore made with a fine needle. The other end of the cylinder is occupied by a perforated indiarubber stopper pierced by a piece of glass tubing. To the tubing is attached a litre flask, which has another tube running from it to a second litre flask, the interior of the two flasks being thus in communication. The cylinder, indiarubber sheeting, and stopper are purified by washing first with corrosive sublimate solution (1 in 1000), and then with absolute alcohol. A quantity of liquefied nutrient jelly

[1] The success of these experiments depends on the facts, demonstrated by Tyndall, that the germs present in any atmosphere constantly gravitate to its lower strata, and that by allowing subsidence to occur for a sufficient length of time, in a closed space, a perfectly pure germless atmosphere is produced.

is now introduced into the cylindrical tube, sufficient to make a thin layer along its floor. The tube leading from the cylinder is clamped, and the pore in the indiarubber sheeting is covered with a cap of sterilised cotton wool. The glass cylinder and its contents are then steamed for thirty minutes on each of two consecutive days, to ensure their sterility, the cylinder being kept level during this process. On

FIG. 47.—Hesse's apparatus for determining relative purity of atmospheres (qualitative and quantitative).
a. Hollow glass cylinder, with (*b*) perforated indiarubber sheeting.
c. Exhausting apparatus, with siphon action.

cooling, the jelly sets in a thin layer on the lower aspect of the cylindrical glass tube. In performing the experiment, the litre flasks, which are filled with water, are put in communication with one another, and with

the tube proceeding from the cylinder. The clamp and cap of cotton wool are removed, and the litre flask more distant from the cylinder is allowed to empty itself by siphon action, drop by drop. Water is thus drawn out of the nearer flask to supply the place of that removed from the other, and air is drawn from the cylinder to take the place of the water. Thus a current sets in from the outer air, through the fine pore, into the cylinder, carrying with it representatives of the germs in the atmosphere tested. A litre of outside air will have been drawn slowly into the cylinder, by the time the litre flask is quite empty, and then the experiment is stopped by clamping the tube and replacing the cap over the pore in the indiarubber sheeting. The germs, which enter by the pore, rapidly gravitate into the layer of gelatine on the floor of the cylinder, and the number of foci of growth which appear in the jelly a few days after exposure corresponds to the number of germs per litre in the atmosphere tested.[1]

(*C.*) Examination of Water for Micro-Organisms.

46. This examination is now looked upon as a matter of considerable importance, and in this country and in Germany various methods have been suggested for making it as complete and as accurate as possible.

Where any sediment can be obtained from the water, after allowing it to stand for some time, it is a comparatively easy matter to examine with the microscope the organisms which are deposited along with the solid particles. Without further amplification, however, such an examination is always far from satisfactory, and affords

[1] Dr. Maddox very ingeniously caused currents of air to impinge on a glass slide smeared with pure glycerine. The glycerine was then examined microscopically. With this glycerine inoculations may be made into various media, in order that the organisms collected may be more thoroughly studied. Dr. Arthur Ransome examined the breath of persons affected with phthisis for tubercle bacilli, by collecting the breath in a glass globe, and condensing the aqueous vapour contained in this breath, by surrounding the globe with a mixture of ice and salt. The condensing vapour, "it was found, carried down all the organic matter contained in the breath," and micro-organisms could be examined in this condensed aqueous vapour.

In order to distinguish the tubercle bacilli, he mixed a small quantity of white of egg with the water so obtained, and then treated the mixture as if it were ordinary sputum, staining by Heneage Gibbes' method. This method may be employed with advantage wherever the air contains a large percentage of moisture.

very incomplete information. Combined with this, the method of staining a dried specimen with one of the aniline dyes, and then preparing by His's method (§ 32, p. 46) is extremely useful. Inoculations with the water to be examined of one of the numerous cultivation media, by means of a fine glass pipette or a platinum wire, should always be made. In the case of spores of certain fungi, bread paste "medicated" with nutrient fluids serves as a capital medium; whilst for bacteria, the transparent solid media answer best, the growth of the micro-organisms being readily distinguished with the naked eye, or by means of a very low magnifying power. The methods now most commonly used are those based on Koch's gelatine method; the first devised by Koch himself, the second devised by the late Dr. Angus Smith.

Koch mixes a definite quantity of water with a certain quantity of warmed peptonised meat jelly. Whilst the mass is still fluid, it is poured on to a level surface, on which the points of growth of the micro-organisms are afterwards readily recognised.

The method of procedure is shortly as follows:—

On a tripod, which, by means of screws, may be raised or lowered at any point as required, is placed a flat glass plate ruled into squares by two series of lines at right angles to one another, and about half

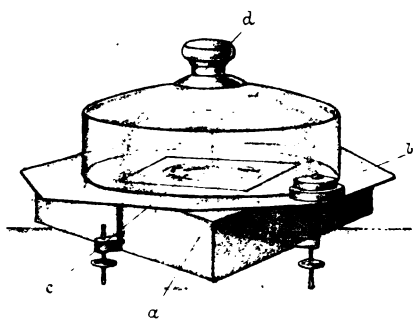

FIG. 48.—Apparatus for determining relative purity of water.
a. Tripod, with adjustable feet.
b. Large glass plate, on which spirit level rests.
c. Smaller plate, with inoculated gelatine in centre.
d. Bell jar.

an inch apart. The centre of this is covered with a bell jar; care having been taken to have both plate and bell jar thoroughly sterilised,

either by heat, or a solution of perchloride of mercury one-tenth per cent. (see Fig. 48). At one corner of the plate is placed a small spirit level, by the aid of which, and the screws on the feet, the frame is adjusted until the plate is perfectly level.

A number of smaller glass plates (of such a size that they will readily go under the bell jar) are then put into a sheet-iron box, with an overlapping lid, and are exposed to a temperature of 170° C. for at least one hour in the hot air chamber. They are then allowed to cool, and, after the operator's hands have been perfectly purified by means of soap and water and perchloride of mercury solution, one of the plates is taken out and rapidly transferred to the tripod on to the larger plate and under the bell jar. A small quantity of the peptonised meat jelly, about 5 c.c., in a sterilised test-tube is warmed in a water bath until it is quite fluid, and then a couple of drops or more of the water to be examined are carefully introduced by means of a fine pipette. (The drops of water must all be of equal size.) The sterile plug of wadding is rapidly returned to the mouth of the test-tube. The whole mass, whilst still warm, is well shaken; then, raising the bell jar carefully, the liquid is poured out on to the small glass plate, and the bell jar is lowered. The measured squares on the larger plate are easily seen through bell jar, upper plate, and layer of gelatine; but, for the sake of convenience, the upper plate may be ruled, especially where a number of samples of water are examined on the same day, and where in consequence the small plates are in turn removed to a larger sterilised chamber or bell jar, where they may be examined from time to time, at first merely for the number and naked eye characteristics of the growths which make their appearance at more or less regular intervals. Inoculations from these growing points may afterwards be made into various nutritive media, bread paste, gelatine, agar-agar, &c.; careful microscopic examinations must also be conducted.

Dr. Angus Smith's modification of Koch's solid medium process serves admirably for the demonstration of the number of micro-organisms. He describes the method of working as follows:[1]—"A solution was made containing five per cent. of solid gelatine.

[1] Dr. R. Angus Smith "On the examination of Waters." Second Report to the Local Government Board, 1884.

This was prepared from the thin leaf, then, when dissolved, clarified by filtration or by fresh albumen. This solution melts at about 27° C., or about 80° F.; 25 c.c. of this have been usually mixed with 25 c.c. of the water to be examined, and kept for some minutes about the

FIG. 49.

FIG. 50.

FIG. 49.—London waters, with 80 per cent. distilled water added to gelatine sugar and sodium phosphate. Appearance presented in the test-tube at the end of five days.

FIG. 50.—London waters added to gelatine sugar and sodium phosphate. Appearance at the end of five days. (Copied from Angus Smith.)

temperature indicated, but much smaller quantities are frequently sufficient."

The water is therefore added to the gelatine in large test-tubes whilst it is still fluid; after which, as it cools, the whole mass becomes

solid, and any organism developing remains with its progeny and its products in one position, and "one sees at a glance the number existing in the water."

In very impure water the organisms which are found in it in great abundance appear to require the presence of oxygen for their growth, and therefore liquefaction of the gelatine takes place only at the surface, and the deeper parts may appear to contain fewer active organisms than some water that is comparatively pure, in which "the whole water becomes filled with perfectly formed and transparent spheres, forming very beautiful objects. At the bottom of the spheres is a little white line; sometimes this becomes rather marked, and if it is too heavy, it sinks below the circumference, and gives an appearance of a balloon with a parachute."

"If a pipette is put into these spheres, and suction applied, it will be found that they are full of liquid, whilst the gelatine around is solid. The deposit below contains a great mass of active and inactive bacteria."

"There is another phenomenon appearing in some of them, viz.—a number of very minute white dots, which increase to a size of a pin-head," but which do not liquefy the gelatine. "These may be scattered in countless numbers through the water, and seem to indicate the number of points of vitality in the water."

Dr. Smith considers that gelatine alone gives sufficiently good results, but that phosphate of soda or sugar, or both, may be added to the gelatine, in which cases the changes go on more rapidly, even too rapidly for accurate observation. This difficulty, however, is easily overcome by taking a smaller quantity of water, so that the spherules may be less numerous.

The great advantage that this process has over the flat glass plate method is, that observations may be carried on in the same specimen for a very considerable period, the large mass of gelatine affording ample space for extension in all directions, in addition to which there is not any great tendency to dry, as there is in the case of the thin film of gelatine on the glass plate. On the other hand, it is impossible to get secondary inoculations on to other media by Dr. Smith's method, though he suggests that part of the surface growth may be diluted with distilled water and added to a fresh quantity of gelatine.

Dr. Angus Smith has also suggested an excellent method "of measuring the amount of organic life existing in the water by means which may be called purely chemical." He measures their activity by their power of evolving hydrogen during the decomposition of sugar, which they bring about. He proceeds as follows:—"Tubes seven and a quarter inches long and three-quarter inch diameter were filled with the water to be examined, to which one per cent. grape sugar was added. These were inverted, sealed at the bottom with mercury, and allowed to stand. In every case there was a control experiment made by using the purest distilled water, to which the same amount of sugar was added." After five days gases commenced to collect, and, in most specimens of water examined, gas was eliminated. He then washed the gas with caustic potash, and, by means of this and pyrogallic acid, found that carbonic acid was present in small quantities only, and that oxygen was absent. The gas was fired with oxygen, and found to be composed almost entirely of hydrogen and nitrogen, and this evolution of hydrogen by the micro-organisms acting on organic matter is made the measure of their vital activity.

(*D.*) Cultivation in Sterile Agar-Agar Mixture.

47. In consequence of the fact already mentioned, that the five per cent. gelatine solution liquefies if its temperature be slightly raised, a more resisting substance has been introduced by Maddox and Koch, which is equal to peptonised jelly in its nutritive qualities and in its transparency, and which will remain solid until a very high temperature (80° C.) is reached. Agar-agar consists of the dried sliced stem of a *fucus*, and is largely used in culinary science under the name of Japan isinglass. If this substance be added in the proportion of one per cent. to the beef-peptone broth used in preparing peptonised jelly, as already described (§ 43, p. 72), and the gelatine be omitted from the mixture, a very nourishing solid medium is produced. Its preparation and sterilisation are completed in precisely the same manner as was described in the case of the jelly, and it is used in the same ways as that substance, over which it has the great advantage in certain cases of being capable of constant incubation, even at high temperatures, without losing its solidity.

(*E.*) Cultivation on Sterilised Solidified Blood Serum.

48. Another solid and transparent cultivating medium is supplied by the serum which is separated from blood after it has clotted. The blood of any animal will supply this, but it is found that that from the calf is most abundant, and has the greatest degree of translucency. Having been sterilised by a long process of interrupted heating, it is solidified by carefully inspissating it at a high temperature. To obviate as far as possible the risks of contamination from without, the blood is carefully collected in prepared jars at the period of slaughtering. Tall stoppered glass jars (see Fig. 51) are prepared for its reception, by being thoroughly cleansed, first with a one per cent. solution of mercuric chloride, and then with absolute alcohol. The stopper is smeared thoroughly with vaseline containing corrosive sublimate, to prevent its becoming fixed, and to exclude atmospheric dust from the jar and its contents. Jars thus prepared are allowed to stand in an ice-press for several hours before use; and, during the process of slaughtering, the blood is allowed to flow directly from the animal's neck into a jar, unstoppered for the moment for this purpose. The first gush of blood is rejected, to avoid impurities which it might acquire in passing through the wound. When the jar is filled to within two inches of the top, it is re-stoppered and placed at rest in an ice-press or cool place for twenty-four hours.[1] At the end of that time, coagulation and the sub-

Fig. 51.—Jar for collecting blood.

[1] During the first few hours, a small quantity of thin and watery but deeply stained fluid accumulates at the surface of the coagulated mass. This should be carefully removed with a sterilised pipette, or it may discolour the serum which collects around the contracting clot, and interfere somewhat with the transparency of the fluid, and still more with the solidified serum.

sequent contraction of the blood-clot are complete, and a mass of fibrin containing all the red blood corpuscles is found floating in a quantity of clear limpid serum, which has been extruded on the contraction of the coagulum. This clear serum must now be transferred

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suction. Hence it is allowed to trickle down into the lower part of one of the sterile test-tubes, which is unplugged to permit of this,

and the plug is replaced immediately afterwards. This process is repeated till a sufficient number of tubes are prepared; and the test-tubes thus charged with serum are at once placed in the "slow steriliser" (see Fig. 52), the temperature of which is raised to 57° C. At this temperature, which must on no account be more than very slightly exceeded, the apparatus is maintained for six hours on the first day of the sterilising process. On each of the five succeeding days the apparatus must again be raised to the same temperature, and maintained there for four hours each day. The

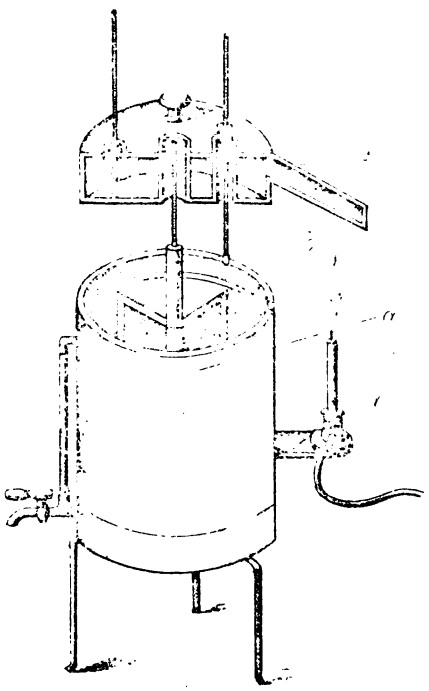

FIG. 53.—Slow steriliser for blood serum.
a. Main chamber with water jacket.
b. Central tube communicating with water in jacket.
c. Lid with diverticulum, heated by Bunsen burner, *d.*
Thermometers pass into central chamber, lid, and water-jacket.

temperature is not allowed to mount higher, lest premature coagulation should occur in the serum, and its transparency should thus be

lost. At the end of this process the serum is sterile, but still liquid. In order to solidify it, without at the same time destroying its clearness and transparency, the tubes containing it are laid on their sides

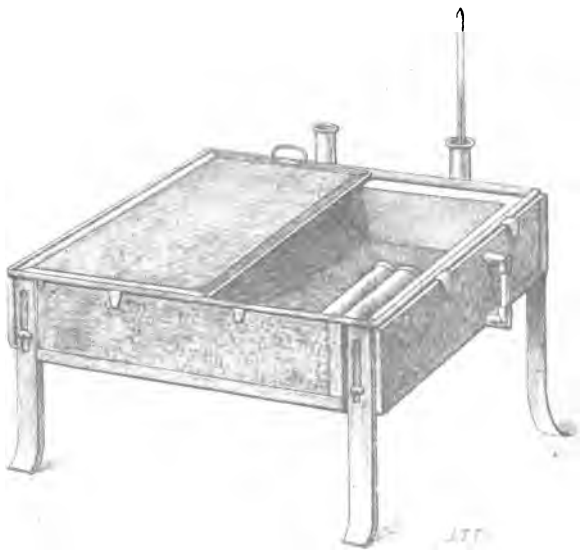

FIG. 54.—Serum inspissator or thermostat, with two test-tubes in position. One-half of glass lid and felt cover are not shown in drawing. The apparatus consists of a double-walled box, covered with felt, and heated from below; the temperature being maintained by warm water occupying the interspace between the two walls.

on the sloping floor of a thermostat (see Fig. 54), which is so tilted that the plane of the surface of the serum cuts that of the lumen of the test-tube obliquely, and the surface of the serum is thus largely increased, and offers a wider area for the cultivation of micro-organisms. This is of the greater importance, from the fact that in the case of most organisms which can be advantageously cultivated in this medium it is found that growth only occurs on the free surface of the serum, and not at all in its deeper layers.

The next part of the process requires the most careful and constant attention of the operator. It consists in raising the temperature in the thermostat to from 68° to 80° C., which causes a more or less rapid solidification of the serum. The serum first becomes solid merely, retaining its transparency, but if the process be allowed to

pass in the least beyond this stage, the transparency is replaced by a dull opacity that becomes denser the longer the serum is exposed to the increased heat. It is necessary, therefore, to remove the test-tubes from the thermostat the moment their contents have become solid, and before the opacity has commenced to appear. If this stage is passed, the solid serum is still a useful nutrient soil, but it has lost that perfect translucency which constitutes its special value, inasmnch as it allows of the exact observation and delineation of the special methods of growth exhibited by special organisms. The amount of heat requisite to inspissate the serum varies in different cases, and chiefly in relation to the various sources from which it is obtained. Thus solidification takes place at a lower temperature in serum from the blood of sheep, oxen, and pigs, and in human pleuritic and peritonitic fluids, than it does in calf-blood serum, or in the fluid from hydrocele. As it becomes solid, a few drops of water are evaporated from the serum and condensed in the tube. In addition to the above-named sources of supply may be named the contents of blisters, and of any of the serous sacs, and the vitreous humour of the calf. Before preparing serum from any of the sources mentioned in large quantities, it is advisable to ascertain the amount of albumen present, by heating a sample of the liquid in a test-tube over the flame of a Bunsen burner. If the serum becomes solid, it will answer well for cultivation purposes, and can be prepared as already described. If only a flocculent precipitate is thrown down, it cannot be prepared thus with advantage, as it will refuse to solidify in the thermostat. Such serum, however, can be used in combination with gelatine as a useful culture medium.[1]

Summary of Process for preparing Sterilised Blood Serum.

First Day.—Collect blood in sterile jars, and allow to stand for twenty-four hours.

[1] Koch has largely employed, and highly recommends, a *sero-gelatine* solution, consisting of limpid blood serum, to which five per cent. of its bulk of gelatine is added, and which must be sterilised by the "slow process" above described. It is employed exactly as is the peptonised jelly. Care must be taken that it is not on any occasion heated above 60° C., when coagulation and opacity would be likely to ensue. (Koch, *Mittheilungen a. d. k. Gesundheitsamte*, Bd. i., p. 27.)

Second Day.—Remove serum with sterile pipette into sterile test-tubes.

In slow steriliser at 57° C. for six hours.

Third Day.
Fourth „
Fifth „
Sixth „
Seventh „
} In slow steriliser at 57° C. for four hours.

Eighth Day.—In thermostat 68° to 80° C., till solid.

Method of Inoculation.

49. To inoculate one of the sterile tubes of serum, a mounted platinum wire of medium thickness is chosen, with sufficient rigidity to enable it to produce a furrow on the surface of the tough serum. The tip of the wire is bent at a right angle (see Fig. 21), and upon it, after it has been sterilised at a white heat, is placed an infinitesimal quantity of the seed material. The test-tube is held inverted, to prevent the entrance of atmospheric impurities; the cotton wool plug is then removed, and held between two of the fingers, with its lower part pointing downwards (Fig. 34), and the loaded platinum wire is now introduced into the test-tube. The bent point of the wire is pressed down upon the surface of the solid serum, and longitudinal furrows are made by drawing the wire in a series of parallel lines from the lower extremity towards the mouth of the test-tube, and in this way the seed material is implanted in linear tracts, along which the characteristic features of the resulting growth may be observed. The wire is now withdrawn, and the cotton wool plug replaced in the mouth of the tube. The wire is immediately heated to a white heat, to obviate the risks of disseminating the seed material, which is often of a dangerous character, amongst neighbouring objects.

The test-tubes may be placed in an incubator, and kept at blood-heat, if this be desirable; and the serum so used has this advantage over peptonised beef jelly, that it retains its solidity at all temperatures.

Inspissated blood serum prepared in this way in sterile test-tubes has been chiefly employed in the cultivation of some organisms which

do not flourish so well in peptonised jelly as in this medium, and notably for the culture of the tubercle bacillus.

FIG. 55.—Tubercle bacillus cultivated upon solid serum in test-tube, glass capsule (after Koch).

FIG. 56.—Tubercle bacillus upon solid serum.

At an even summer temperature the tubercle bacillus flourishes admirably in this soil, and presents a quite characteristic appearance. It grows in the form of a delicate white pellicle (see Fig. 55), which has a flaky, almost fluffy, appearance, and a dull granular surface, which forms a marked contrast to that of the solid serum, which is smooth and shining. This pellicle covers the whole of the surface of the serum in course of time, and forms a thin membrane over the few drops of moisture which occupy

the lower part of the test-tube. It in no way modifies the clearness of the liquid, nor will it mingle with it, but is torn up and washed away in flakes, should the water be allowed to trickle over the surface of the serum. The same organism can be advantageously cultivated in sterile capsules of solidified serum (see Fig. 56), when it presents similar features, which can here be studied to even greater advantage than when it is grown in test-tubes.

CHAPTER IV.

LIQUID CULTIVATING MEDIA.

50. In addition to the media described in the preceding chapter, there is a long series of fluid media used in the cultivation of micro-organisms. They are of two main types—firstly, those obtained by infusing animal or vegetable substance in water; and, secondly, those produced by the artificial commingling of the chemical elements which are present in the bodies of micro-organisms, and which are hence necessary constituents of their food. In some of the latter the quantities of the different constituents have been graduated with exquisite precision to the requirements of the organisms that it is desired to cultivate; whilst in the former, where fully elaborated natural products are employed, in which doubtless a similar exactitude of molecular relations exists, the requisite food material may be obtained by a less complex synthetical process.

As compared with solid media, three points must be noticed in relation to the growth of micro-organisms in fluids. In fluid media, organisms grow more rapidly than in solid. In the former, however, they do not, as a rule, exhibit such distinctive specific features as in the latter; but in fluid media there is an opportunity of gauging exactly the chemical requirements and relations of micro-organisms, which does not exist in the case of solids. For the study, then, of the physiological peculiarities of any organism, one would choose a fluid nutrient material; whilst for that of its morphological relations, solid media would be much better adapted.

Animal Infusions.

51. Fresh meat (beef, pork, veal, chicken, &c.), free from fat, and, as far as possible, from fibrous textures, is finely minced and extracted with cold distilled water for twenty-four hours in a cool place, a litre

of water being added to a pound of the meat. From time to time during this process the whole should be well stirred. It is then filtered through a piece of fine linen cloth, and the pulpy mass is subjected to pressure in the screw press (see Fig. 31), to extract as much fluid as possible from it. The broth thus obtained is usually distinctly acid (see § 43, p. 73), and must be neutralised by the addition of a saturated solution of carbonate of soda, drop by drop, until the broth becomes quite neutral in its reaction.

It is now received into a glass flask (one litre), and is placed in the steam steriliser at 100° C., and steamed (not boiled) for thirty minutes. A fine precipitate is found to occupy the lower layers of the flask after this process. This is got rid of by filtering the broth through a filter which has immediately previously been washed thoroughly with boiled distilled water, and the clear filtrate is received into stock flasks (quarter litre), which, with their plugs, have been previously sterilised by superheating (see § 43, p. 75). The stock-flasks thus charged are steamed for thirty minutes in the steam steriliser at 100° C. on each of two consecutive days, standing in a cool place in the intervals of steaming. The stock-flasks are incubated for four days at 32° C., and if no reaction occur, they may be kept as a reservoir of sterile material for future use in cultivation.

To carry on cultivations with the sterile broth thus prepared, it is convenient to have it divided into small quantities. For this purpose a number of one ounce Bohemian flasks are prepared, supplied with cotton wool plugs, and sterilised by superheating in the hot air chamber (Fig. 33); and 10 c.c. of the sterile broth is transferred to each flask by the use of a superheated glass pipette, as described under "nutrient jelly" (§ 44, p. 78). This should either be carried out under the carbolic spray (footnote, p. 78), or, when charged, the small flasks should be steamed at 100° C. on two successive days, for fifteen minutes each day.

Inoculations are carried out by means of a platinum wire as already described, with the exception that here the flasks cannot be inverted to avoid atmospheric contamination, and in this case it is best to inoculate with seed material, either under the spray, or under the protection of a canopy of cotton cloth soaked in a one per cent. solution of perchloride of mercury. Any of these infusions may be rendered

more suitable for the growth of organisms, by the addition of one per cent. of albumen peptone before the initial neutralisation and filtering are carried out.

Buchner's Fluid.

52. Amongst the nutrient animal infusions, that recommended by Buchner will be found of great value. It consists of—

Liebig's meat extract, . . .	10 parts.
Albumen peptone, ...	8 „
Distilled water, ...	1000 „

Before it is used it must be sterilised and incubated under observation for a week, as has been already described.

A similar peptonised liquid can be even more easily procured by using Darby's fluid meat (peptonised) in a half per cent. solution :—

Darby's fluid meat, ...	5 parts.
Distilled water, ...	1000 „

Sterilise by steaming, and incubate under observation before use.

Urine.

53. Urine passed into sterile flasks with antiseptic precautions (use of spray, careful purification of meatus) does not become alkaline, as it would do if exposed under ordinary conditions to the air. This is equivalent to stating that it remains sterile; and it forms, without further preparation, an admirable soil for several kinds of fungi (moulds); but from its slightly acid reaction, it is not, in the first place, so well adapted to the growth of the cleft fungi (bacteria). Should, however, one of the latter (*Micrococcus ureæ*, Cohn) once establish itself in the urine, it gives rise to the ammoniacal fermentation, and prepares the way for the growth of its congeners by rendering the liquid alkaline; whilst the moulds, overpowered by the rapid growth of cleft fungi, soon lose their vitality, and are disintegrated. If urine cannot be collected with the precautions named, it should be placed in sterile flasks, and treated as any of the animal infusions above described, before it is used for experiments.

Milk.

54. If collected in sterile flasks with careful aseptic precautions (pure air, or spray, and careful purification of teats [1]) milk will remain

[1] "Antiseptic Surgery," Watson Cheyne, pp. 38-39, 1881.

quite sterile, and may be used as a culture medium for a great number of organised species (torulæ, bacilli, micrococci). As obtained from ordinary sources, milk must be thoroughly sterilised before use. Its sterilisation is more than usually difficult, from its physical character as a bad conductor of heat, and from the fact that it is early contaminated with germinal matter in the usual methods of its storage and transmission. This germinal matter consists, amongst other elements, of the dried spores of *Bacterium lactis* (Lister) and *Bacillus butyris* (Pasteur, Cohn), which, when brought into contact with fresh milk under suitable conditions of temperature, &c., rapidly take on vegetative activity, and render it difficult of subsequent sterilisation. The method of destroying this activity, and rendering the milk sterile, is by steaming that liquid repeatedly for thirty minutes at a time, at intervals of twenty-four hours, for five or six days. If it be only twice steamed, as prescribed for other animal liquids, *B. lactis* is usually excluded, but after an interval it is often found that the cream layer has become rancid, owing to the development of *B. butyris*, although the milk beneath that layer may remain quite pure. When thoroughly sterilised, milk should be incubated at 32° C. under observation for a week, and if it remain unaltered, it may then be used as a culture medium.

Blood Serum.

55. Limpid serum is obtained from the various sources (blood from slaughter-houses, hydroceles, pleuritic and peritonitic effusions, blisters, &c.) already mentioned when treating of the solid media (see § 48, p. 102). It is sterilised by the slow process as previously described, and it can be used as a culture medium in the various ways enumerated under "animal infusions."

Cheese Infusion.

56. Infusions which contain cheese as one of their constituents must be sterilised with the greatest circumspection, and afterwards subjected to the test of prolonged incubation. This is rendered necessary by the great resistance which cheese offers to the sterilising process. The quality of impermeability by heat,[1] which it possesses in common with milk, accounts for this difficulty in effecting its sterilisation.

[1] Tyndall, *Proc. Roy. Soc.*, 1877, vol. 26, p. 228.

Vegetable Infusions.

57. Watery infusions of hay, turnip, tobacco, &c., which have been largely used in the cultivation of many forms of micro-organisms, are prepared by macerating the several vegetable substances employed in distilled water for eight hours, and then raising the temperature gradually to the boiling point. The infusions are then filtered, transferred to sterile flasks (previously superheated), and sterilised by the method already described under "Animal Infusions" (§ 51, p. 107). They may be made of varying degrees of concentration, according to the requirements of various organisms, and the luxuriance of growth that is wished for.

Sterile grape juice is obtained by preparing the filtered juice of ripe grapes in the same manner. It affords special facilities for the growth of *Torulæ* and the *Mycoderma aceti*.

Artificial Solutions.

Raulin's Fluid.

58. A mineral solution, made up of elements the exact relative proportions of which were determined by the results of chemical analysis of the tissues of the group of micro-fungi (*Aspergilli*), for the cultivation of which it was devised. It consists of—

Distilled water,	1500 parts.
Cane sugar,	70·0 ,,
Tartaric acid,	4·0 ,,
Ammonium nitrate,	4·0 ,,
Ammonium phosphate,	0·6 ,,
Potassium carbonate,	0·6 ,,
Magnesium carbonate,	0·4 ,,
Ammonium sulphate,	0·25 ,,
Zinc sulphate,	0·07 ,,
Ferrous sulphate,	0·07 ,,
Potassium sulphate,	0·07 ,,

The employment of this solution has been productive of a great increase of knowledge as to the intricate chemical relations and food requirements of the group of the moulds. It may be used without

modification for their cultivation; and various slight alterations in its composition render it of great value in the culture of several of the other groups of micro-organisms.

Pasteur's Fluid.

59. Pasteur's fluid consists of—

Distilled water,	1000·0 parts.
Pure cane sugar,	100·0 ,,
Ammonium tartrate,	10·0 ,,
Ash of yeast,	0·75 ,,

This formula has a special interest attaching to it, as it is that of the first synthetically prepared medium employed in the cultivation of micro-organisms.

Cohn's Fluid (as most recently modified.)

60. This consists of—

Distilled water,	200 parts.
Tartrate of ammonium,	20 ,,
Phosphate of potassium,	20 ,,
Sulphate of magnesium,	10 ,,
Tribasic phosphate of lime,	0·1 ,,

Miquel's Fluid.

61. Whilst disparaging the use of Liebig's extract of meat as a nourishing medium, Miquel[1] recommends an infusion prepared according to the following formula :—

Boil 1 kilogramme of lean beef in 4 litres of distilled water, for five hours.

Strain and neutralise with caustic soda solution. Add 40 grammes of common salt (10 gr. per litre).

This infusion should be sterilised by discontinuous steaming in stock-flasks of convenient size, and incubated thoroughly before using it as a cultivating medium.

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[1] "Les organisms vivants de l'atmosphere," p. 152.

Lister's Flask.

62. A convenient method of storage for sterile liquids consists in making use of Lister's flasks (Fig. 57). They consist of spherical

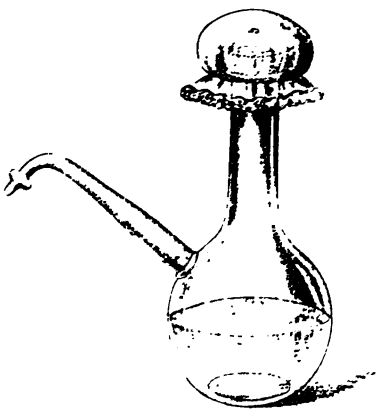

Fig. 57.—Lister's Flask.

Bohemian glass flasks with two necks—a vertical and a lateral one; the former is straight, and is plugged in the usual way with sterile cotton wool (§ 41, p. 64); the latter consists of a bent tube, piercing the side of the flask, and ending with a constricted neck in a tapering nozzle. This lateral entrance is protected by a mass of sterile cotton wool enclosed in a piece of gauze, and secured to the constricted neck of the tube by a piece of twine or an elastic ring. These flasks, which contain about half a litre each, are filled only so full as to avoid the risk of their contents reaching either of the plugs during the process of ebullition, in which case the clearness of the liquid might be lost by the abstraction of dust or fibrils from the cotton wool of which the plugs are made. When it has been charged with a quantity of stock liquid, the Lister's flask is sterilised by subjecting it to heat, that of the steam steriliser (Fig. 14) being preferred, from its preventing evaporation during the sterilising process. The stock liquid can then be kept for any length of time without becoming contaminated. When it is desired to abstract some of the contents of the flask, the sterile pad is removed from the bent lateral neck, and as much liquid as is required is allowed to flow from the lateral

aperture by tilting the flask to one side. On restoring the flask to its upright position, it is found that though the liquid at once gravitates back from the lateral bent tube, yet there is no regurgitation of the mote-bearing air from without, which would almost inevitably contaminate the sterile liquid. This is prevented by a drop of liquid that occupies the extreme end of the lateral tube, which it completely occludes, and thus prevents the air from passing in. After a quantity of the liquid has been withdrawn, a fresh sterile pad is placed over the mouth of the lateral tube, and fastened in that position as above described, and the residue of the stock liquid can be maintained sterile for an indefinite period.

AITKEN'S TEST-TUBE.

63. This supplies a very convenient means of carrying on pure cultivations in liquid media. The tube (Fig. 58) has a lateral diverticulum in the form of a narrow glass tube, which tapers and ends in a fine impervious point. The test-tube is plugged with cotton wool, and sterilised by superheating. It is then charged with 10 c.c. of sterile culture fluid, and incubated for four or five days. If its contents remain unaltered, it may be inoculated for a cultivation experiment. To do this the seed material is first prepared on the tip of a sterile platinum wire. The end of the lateral tube is then snipped off with a pair of sterile (superheated) pliers, and the wire, introduced carefully along the lateral tube, deposits the seed material upon the inner wall of the test-tube, opposite to the point whence the diverticulum springs. The wire is then withdrawn, and the end of the lateral tube sealed by fusing it in

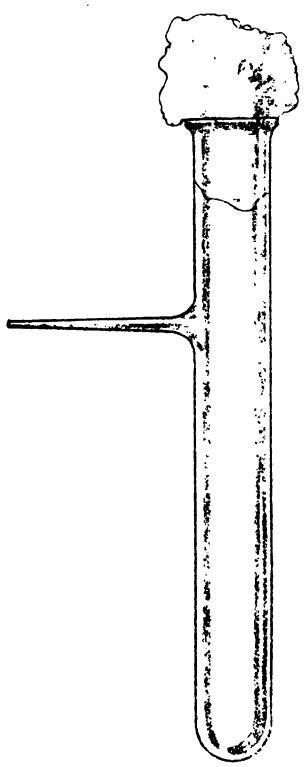

FIG. 58.—Aitken's Test-Tube.

the flame of a Bunsen burner. The test-tube is now tilted so that its liquid contents come in contact with the seed material deposited on its wall, and the inoculation is thus ingeniously provided for, with absolutely no fear of contamination. There is no tendency for germs to be wafted in from the outside atmosphere during the inoculation, as the sieve-like action of the cotton wool plug provides for the atmospheric pressure being equal within and without the test-tube.

Sternberg's Bulb.

64. Sternberg has invented another method of making pure cultivations in liquid media. It consists in the use of a very small glass bulb with a tube opening into it. The bulb (see Fig. 59) is charged

Fig. 59.—Sternberg's Bulb.

by first heating it; the tip of the tube is then broken off, and the end of the tube immersed in the culture liquid. As the bulb cools the liquid is drawn up into it, and usually fills about one-third of its cavity. The tube is now sealed up again in the flame of a Bunsen burner, and several bulbs thus charged are sterilised by intermittent heat in a water or sand bath. They are then incubated for several days, and if they then show no signs of contamination, they may be used in carrying on cultures. In inoculating them the bulb is heated, the tip of the tube broken off, and its end immersed in the material that it is desired to cultivate. A trace of this at once enters the tube, and becomes mingled with the liquid in the bulb. The tube is again carefully

sealed. In this way separate cultures of isolated organisms are obtained, and their characteristics may be observed with great ease in this form of cultivation.

INCUBATION.

65. Incubation of culture media which have been inoculated should be, as far as possible, carried on at even temperatures. To maintain any temperature equally for a length of time, one or other form of carefully constructed incubator is absolutely necessary. That depicted in Fig. 60 is a convenient form, from its having double glass sides,

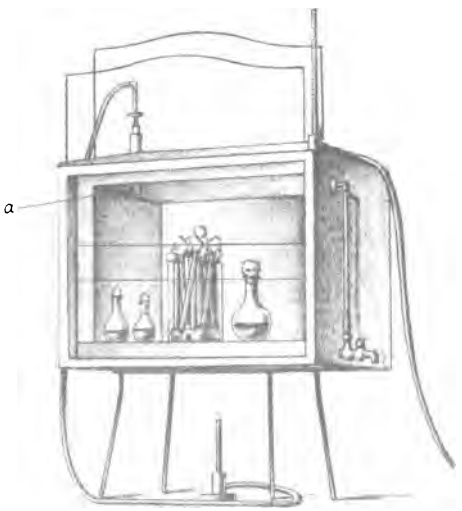

FIG. 60.—Incubator, with regulator (*a*) in air chamber.

which allow of the contents being from time to time examined, without removing them from the warmer atmosphere within the apparatus. The apparatus is double-walled, and the interspace between the walls is occupied by a layer of water. There is, of course, a layer of air between the double glass walls. The heating is carried on by a small Bunsen burner in connection with a gas regulator, which controls the amount of gas supplied to it. The tube of the regulator lies in the water, within the hollow walls of the incubator, or in the air chamber, and is so constructed that when the temperature of the

water or air rises high, a column of mercury in the regulator rises also to such an extent as to cut off nearly all the gas supply which passes through the regulator to the burner. In this way the flame is reduced, and the temperature of the water begins to fall; as it does so, the column of mercury falls, and more gas is permitted to pass. By these two processes of mutual accommodation it is usually easy to maintain a very equable temperature in the incubator. To cultivate many species of organisms successfully it is necessary to incubate the media in which they are. Generally speaking, a temperature of from 30° to 36° C. is the most advantageous for inducing a rapid development of the cultivated organisms.

CHAPTER V.

SEPARATION OF MICRO-ORGANISMS FROM THE TISSUES.

66. In order to carry out pure cultivations, as described in the preceding chapters, it is essential to be able to separate the seed material which it is desired to cultivate, and inoculate it in suitable media uncontaminated by foreign elements. Some differences of method must be noticed in this respect, according as the tissues supposed to contain organisms are living or dead.

1.—From Living Tissues.

67. (*a.*) *Blood.*—In the case of the human subject this is usually taken from the finger or from the ear. In either case the skin is first cleansed with warm water and soap, then purified with a one per cent. solution of perchloride of mercury, and lastly washed with absolute alcohol. In the case of the finger, the venous return is hindered by the application of an elastic band, and vascular engorgement of the part ensues. The sterile surface is then pricked with a needle that has previously lain for several minutes in absolute alcohol, and the drop of blood which exudes is collected on the looped end of a sterile platinum wire, and can be at once used for inoculation as already described. In animals the same method may be employed, first shaving the part to be put into requisition; it is more usual, however, to kill the animal, and obtain blood before coagulation has set in, as described in the next section.

(*b.*) *Solid Tissues.*—Here the best method is to make use of Duchenne's needle, which has been previously thoroughly sterilised by superheating (see § 68, p. 118). The skin surface through which it is introduced is sterilised beforehand, as already described (*vide supra*), and the minute portion of tissue thus obtained is at once transferred to the tip of a sterile platinum wire, inoculated into a suitable nutrient medium, and, if need be, incubated.

The same method may be used in the case of animals, but it is usually better to kill them, and obtain the seed material as described under "dead tissues."

(*c.*) *Discharges.*—This comprehends contents of abscesses, and the excretions from glands (*e.g.*, kidney) in which living organisms capable of cultivation may be present. An abscess should be opened with antiseptic precautions (spray, skin purified, &c.), and a drop of pus as it emerges collected on the end of a looped platinum wire, and at once plunged into some sterile nutrient medium. Urine should be collected with similar precautions in a sterile flask, and after cooling, some of the deposit drawn into a newly made glass pipette, and transferred to some nutrient medium. Usually the organisms thrown off in the urine are dead. They can be recognised microscopically, but rarely cultivated. An ingenious method of separating the *Bacillus leprœ* from the living tissues has already been described (see footnote, p. 38).

2.—From Dead Tissues.

68. Unless taken immediately after death, dead tissues are in general useless for employment as seed material. If they can be secured at once, however, pure cultivations of the micro-organisms which they contain may be obtained with ease. In the case both of man and animals a certain amount of dissection must be carried out with aseptic precautions to obtain this result. In making this dissection only sterile instruments must be employed. All the instruments necessary (several knives, scissors, forceps, Pravaz' syringes, &c.) are thoroughly cleansed, and wrapped in sheets of cotton wool. They are then placed in the hot air steriliser for an hour at 170° C.,[1] not heated in the flame, as has been suggested, and which is a most extravagant method, as it destroys the temper of steel completely. They are wrapped in cotton wool, to prevent access of atmospheric germs to the sterile instruments, when the outside air regurgitates into the hot air chamber on its beginning to cool. They are only unwrapped from their cotton wool protection immediately before use.

[1] The knives must have bone handles, as, if made of wood, they become incinerated.

(*a.*) *Blood.*—In the human subject this should be taken as soon after death as possible, by sterilising a portion of the skin, as already described, and introducing the sterile needle of a Pravaz' syringe into a vein from which the blood is withdrawn. This blood can be at once inoculated into various media, and the cultivation of its contained organisms at once proceeded with. In animals the pericardium is laid open with a sterile knife and forceps, and the left ventricle opened with sterile scissors; and then a sterile platinum wire is introduced into its cavity, and some of the blood removed for inoculation.

(*b.*) *Tissues.*—In the human subject either a dissection is made with sterile instruments immediately after death, or a harpoon is employed, as described under living tissues, and a small portion of tissue obtained in either of these ways is planted in a nutrient soil, as already detailed. In animals small portions of tissue are separated by dissection, removed with a sterile platinum wire, and forthwith implanted in a nutrient soil.

In the case of the tubercle bacillus a pure cultivation was first obtained by pounding down a caseous bronchial gland in boiled distilled water, and planting the extract thus obtained in blood serum.[1]

(*c.*) *Discharges.*—It is difficult to obtain pure cultivations of microorganisms from discharges collected after death, as the *post mortem* contaminants are usually both numerous and varied. If the attempt be made, some of the discharge should be diluted with boiled distilled water, and then allowed to trickle in a thin layer over the surface of solid nutrient media (potatoes, gelatine), and the various foci of growth which result may then be separately examined, and any of special interest separated, and cultivated apart in sterile media.

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[1] *Vide* Koch, *op. cit.*

APPENDIX A.

THE following short description of several of the common species of fungi and other micro-organisms met with in the course of ordinary laboratory work, will, we hope, prove of value.

No attempt is made to give a complete classification or *resumé* of known species, which will be discussed at length subsequently. The means of their recognition here supplied will aid the investigator in the detection and exclusion of possible contaminants.

The naked eye appearances of the growths, as seen on or in various nutritive media, and their microscopic characters, will be shortly detailed in the case of each.

PENICILLIUM GLAUCUM.

NAKED EYE APPEARANCES.

This is the commonest form of cheese mould, and is recognised when growing on cheese by its delicate blue colour and soft fluffy appearance. Its spores are constantly present in the atmosphere in such large numbers that it is one of the most frequent causes of contamination in cultivations. It will grow on the most varied substances, and the rapidity of its growth enables it to overpower and exterminate other growths with which it comes in contact. It prefers free surfaces for its growth.

On Potato.—It forms a velvety blue-green covering, dotted over here and there, if the soil be moist, with spherical drops of clear fluid, which are exhaled by the fungus. If the surface of the growth be touched, a fine grey powder of spores, like the down from a butterfly's wings, adheres to the surface with which it has come in contact.

On Bread Paste.—The growth is similar, but a little more luxuriant, *i.e.*, the moss-like layer of fungus is thicker than when growing on potato.

In Jelly.—On surface, as in two preceding soils (Fig. 46, p. 89). It forms a blue pellicle, which floats on the layers of gelatine immediately below it, which in the course of time has become liquid. *In centre*, as small nodules, with fine silky rays proceeding from them in all directions (Fig. 43, p. 87).

In Liquids (*Urine, Broth, &c.*).—As isolated spheres, each the product of a single spore. These spheres are soft delicate globes lying in the lower layers of the liquid, often of large size, and of a semi-transparent grey tint.

Microscopic Appearances.

Mycelium.—Branching fibrils combined into a dense network. This both in liquid and solid media.

Spores.—At the end of the branches of mycelium, *on free surfaces.* Spherical spores are produced in small pyramidal clusters by the transverse division of the terminal twigs, into which the branches break up at their ends. Spores are not formed except on free surfaces; they are never found in growths from liquids, or from the central portions of gelatine cultures. They are round, comparatively large (7 μ.), and highly refractile. They take on aniline dyes easily, as does also the mycelium.

GENUS ASPERGILLUS.

Several common species are included under this genus, and the generic characters which unite them are the nature of the mycelium and the nature of the fructification. The *Aspergillus* head, bearing the spores, consists in all cases of a central club-shaped stem, upon the surface of which the spores are placed. Slight differences in the shape or colour of these basidia, and of the spores which they support, determine the names and characters of the species into which the genus is divided.

A. albus (Fig. 24, p. 65). *On Potatoes, Bread, Cheese, &c.*—A pure white woolly looking growth, forming rounded masses, with a very convex surface.

In Gelatine.—On surface grows as on bread, but does not form so typical a hemisphere as in that substance, the growth being flattened on the surface, or even in some cases slightly concave. It does not liquefy gelatine. In the centre of the jelly it forms spherical nodules of a dull opaque colour, with a distinct limit to their growth peripherally.

In Liquids.—Found as rounded masses at bottom of beaker.

Microscopically.—It has the characteristics of the genus above described, both basidia and spores being quite free from pigment granules. Its generally distinctive feature is its intensely white colour.

A. repens (Figs. 25 and 26, p. 67). *On Potato, &c.*—A sage-green growth with a velvety surface, growing rapidly as a flat field over the surface of the nutrient soil, and forming a layer not more that 0·25 cm. thick.

In Gelatine.—At surface forms colour due to spores; in centre of jelly grows as rounded white nodules, in which no spore formation occurs.

In Liquids.—Floats as green layer on surface.

Microscopically.—Like other species, except that in connection with the spores there are fine granules of the green pigment. The pig-

ment is not within the spores, but forms a powder, amidst which they lie. Mycelium forms an interlacing network, as in other species.

A. FUMIGATUS. *On Potato, &c.*—A slate-blue coloured growth, rapidly extending as a thin paper-like pellicle over the surface of the nutrient soil. If its lateral extension be checked, the layer becomes crenated, being thrown into a series of irregular deep furrows and ridges. Its colour deepens with age to a bronze brown.

In Gelatine.—Forms coloured nodules at surface, colourless spherules in centre of jelly; in the latter no spores are produced.

In Liquids.—Floats on surface as a slate-blue velvety layer.

Microscopically.—As other species, except that fine granules around spores have a slate-blue colour.

A. GLAUCUS.—Precisely similar to above, except that on solid media it does not form a crenated layer, but remains flat; is soft and velvety, and of a light brown colour. The colour due to powdered granules on the basidia, as in other species.

A. FLAVESCENS.—Only differs from above in possessing a light yellow colour, tinged with light green, and its surface, when grown on solid media, becomes gently undulating.

A. NIGRESCENS (Fig. 28, p. 69.)—A growth of a deep rusty black colour; surface velvety, not crenated, when grown on solid media. Colour due to dark brown pigment granules, lying as fine powder around spores. Pigment soluble in ammonia. Other characters as in three preceding species.

A. FULVUS.—Growing precisely like *A. glaucus*, but of a coffee colour.

GENUS MUCOR.

A group of species having a branched, colourless mycelium, the terminal twigs of which carry spherical sporangia. These sporangia are hollow receptacles, in which the spores are contained, like small-shot in a bomb. Differences of species are recognised by the colour of the sporangia, and by some peculiarities in the mode of growth of the mycelium. From a large list of known species the following are selected as those most commonly met with :—

MUCOR MUCEDO. *On Potato, &c.*—A silky white growth, consisting of a tangled tuft of glistening hairs, attaining a height of from one to one and a half inches. At the ends of these fibrils are rounded glistening bodies, at first white, but soon becoming brown, and then an intense black. These are the sporangia.

In Gelatine.—Grows rapidly throughout jelly as a glistening network of fibrils, the typical black sporangia only being produced when it reaches the surface.

In Liquids.—A floating network of fibrils, with a very few twigs raised above the surface, on each of which is borne a sporangium.

Microscopically.—Sporangium, when ripe, seen to be delicately furred on surface; when still unripe, it is quite smooth. Spores densely packed within, can only be seen by bursting sporangium. Pigment amongst spores as black powder, and in texture of capsule, not however in spores themselves. Mycelium consists of greatly elongated cells, the septa between which can be seen.

M. LICHTHEIMII.—Like *M. mucedo* in all respects, except that its growth is not so luxuriant, the mycelium is more delicate, and its fibrils more attenuated. The sporangia are smaller than in *M. mucedo*, and are not pigmented. (Fig. 23, p. 64.)

M. STOLONIFER.—Exactly like *M. mucedo*, except that the mycelium grows in the manner of stolons, shooting into air, and then bending down, and again entering the nutrient soil. Sporangia black, as in *M. mucedo*.

SALMON FUNGUS.—*Saprolegnia ferax*, an aquatic fungus, which grows most readily on dead flies or dead fish, especially if they are placed in running water. It forms a delicate cloud-like mass (an aquatic mucor) on the surface, and grows very rapidly. Under the microscope are seen tubular branching hyphæ, the protoplasmic contents of which are coarsely granular. Reproductive organs elongated, club-shaped zoosporangia, which open laterally or terminally, and rounded oogonia, in which are oospores. The zoospores which escape from the zoosporangia are usually ciliated or tailed.

The growth does not appear to pass deeply into the tissue of its host.

Closely allied to this species are the *Achlya polyandra* and *Achlya apiculata*.

GENUS PENOSPORA.

PENOSPORA INFESTANS is the most important member of this group, which contains nearly forty known species. It is the cause of the potato disease. The full-grown fungus consists of a branched mycelium 0·005 millimetre in thickness, upon the terminal twigs of which oval conidia are supported. The mycelium and conidia produce together a thin grey pellicle, which adheres firmly to the diseased parts of the plant, notably the green stems and the under surface of the leaves. The diseased portions of the plant itself become withered and brown. The mycelium spreads far and wide through the plant, and soon reaches the tubers. In this position it remains during the winter, and, growing up within the young shoots of the new plant, develops once more its mature conidia upon the free surfaces of its host. The spores, falling to the ground, become actively moving swarm-spores, and attack other plants with which they come in

contact. This parasite should be looked for on the under surface of the youngest leaves, where the brown patch, with grey pellicle, forms the characteristic feature of its presence.

GENUS TORULA.

The members of this genus come under the heading of the "sprouting fungi," and have thus much in common, that their mode of multiplication consists in a budding of daughter cells from the protoplasmic contents of the parent cell, and their separation and free growth as individual plants. In their adult forms all consist of rounded or oval cells, with a distinct limiting membrane and soft protoplasmic contents.

TORULA CEREVISIÆ.—*In grape juice*, appears in two forms. *Low yeast* forms a fine amorphous deposit of a yellow grey colour in the lower layers of the liquid. *High yeast* forms a layer of neutral tinted matter close to the surface of the liquid, and this layer has more coherence than is the case with the lower yeast. Each layer consists of aggregations of oval cells; the lower layer being made up of separate cells, the cells of the upper layer being in more intimate organic connection with one another. The cells are oval, and 8 to 9 μ. in length; either separate, or in short branching chains. In some of the cells are contained three or four spores, each 4 or 5 μ. in diameter. The plant will grow freely in saccharated solutions, or in Pasteur's fluid.

PINK TORULA. *On Potato, &c.*—Grows as a rose-coloured succulent film, if the soil be moist. On bread paste, if not very moist, it has the appearance of pink coral (see Fig. 16, p. 59).

In Gelatine.—It grows most freely at the surface, where it produces its characteristic colour. It grows below the surface to a less extent, following the line of the inoculating wire, and in this position it assumes a grey tint, with a slight shade of pink in it (Fig. 39, p. 84).

Microscopically.—It consists of rounded or slightly oval cells 5 to 8 μ. in diameter; and it is found that the pigment is here present in the cells themselves, each of which has a delicate yellow tint, so that when aggregated in large numbers the prevailing pink colour is produced.

The spores of this organism are very widely disseminated, and are so universally present in the air that it forms one of the most frequent atmospheric contaminants of pure cultivation (Fig. 46, p. 89).

BLACK TORULA. *On Potato, &c.*—Grows as a dull sooty crust, with a dry, slightly furrowed surface. On bread paste it has a similar appearance (see Fig. 30, p. 71).

In Gelatine.—It grows chiefly at the surface as a black heaped-up mass, and to a less extent in the track of the inoculating needle, where it forms small black nodules.

In Milk.—It forms a floating black crust, with a dusky grey tint on its upper surface. The milk itself becomes of a muddy colour from an invasion of the deeper layers by colonies of the organism.

Microscopically.—Like *Pink Torula*, but with a dark brown pigment.

MYCODERMA ACETI. *In Grape Juice.*—The vinegar plant appears as a floating film, which rapidly covers the whole of the surface of the liquid. It is a typical aërobe, and cannot live in the lower strata of the liquid. This necessity of oxygen permits of its exclusion from beer vats, the empty air space at the surface being in this case filled with carbonic acid gas, which inhibits the growth of the plant. The film is either corrugated to a very great extent, or it may be quite smooth, depending on the exact species present.

Microscopically.—The film is found to consist of immense aggregations of elliptical or cylindrical cells 6 μ. long by 2 or 3 μ. broad, arranged in irregular branching chains. Multiplication, as in the *Torulæ* proper, by buds and by spores, which appear in the central portions of the cell contents.

M. aceti will also grow feebly on hay infusion, but not on the animal infusions.

SARCINA. *In Milk.*—Causes a dense coagulum, which sinks in irregular masses to the bottom of the whey, which replaces the sterile milk.

Microscopically.—Three species are recognised :—

S. ventriculi.—Round cells in clusters of four or multiples of four. Cells about 4 μ. in diameter, with cell contents frequently pigmented yellow or light brown.

In the stomach they do not form such large aggregations as in artificial media (*e.g.*, milk), the layer colonies being probably broken up by mechanical unrest.

S. urinæ.—Cells very small, 1 μ. diameter, mode of growth and reaction in milk similar to those of *S. ventriculi.* Found in fresh urine.

S. hyalina.—Round cells, 2 μ. diameter, forming clusters which lie in a hyaline matrix. Found in ditch water.

BACTERIDÆ.

Short cylindrical freely moveable cells, with or without flagella ; in some cases alternating with an intermediate stage, in which the immobile organisms are collected together into zooglea masses.

BACTERIUM TERMO. *On Potato.*—It forms a dusky grey glutinous layer, covering rapidly the whole of the cut surface. At the edges, where the process of multiplication is most rapid, the film has a corrugated appearance.

In Gelatine.—Within twenty-four hours this rapidly growing organism has produced a dimple-like depression in the surface of the gelatine at the point of inoculation. This shortly afterwards becomes a funnel-shaped cavity filled with a turbid liquid, in which a faint tinge of opalescent green is evident. By the third or fourth day the upper strata of the jelly are liquefied, and the liquid possesses a bright iridescent green colour (Fig. 44, p. 87). Within a week or ten days the whole of the nutrient jelly has liquefied, and the green gradually gives place to a deep citron colour in the liquid. Grown in jelly, *B. termo* produces little or no odour.

In Liquid Media.—B. termo produces a dense turbidity, equally disseminated throughout the liquid. Its growth is accompanied by a very penetrating putrescent odour.

Microscopically.—In its freely moving form *B. termo* appears as a small dumb-bell, about 1 μ. long and 0·5 μ. broad. Long flagella attached to each end have been described by Dallinger. The organisms have an irregular flickering movement, appearing to rotate on their own long axes, and gliding alternately in either direction, usually in straight lines, but at times describing curves or looped figures in their onward progress.

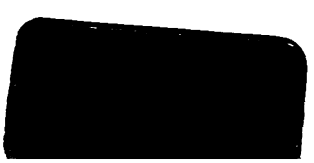
In its zooglea form the bacterium appears as short straight rods, clustered together in a clear transparent jelly-like mass, many of the organisms undergoing division by transverse fission. In this stage the organism lies quiescent, and the flagella are absent. The zooglea mass often forms an iridescent scum on the surface of decomposing liquids.

BACTERIUM LINEOLA. *On potato* this organism forms a film identical in appearance with that produced by *B. termo*, but tending to grow rather more rapidly and luxuriantly. In jelly and in liquid media, there are precisely similar reactions to those produced by the presence of *Termo.* It is only by its microscopic characters that *B. lineola* can be differentiated. In its freely moving form it is elliptical in shape and about 5 μ. long by 2 μ. broad; it also has two flagella, one at either end, and moves in the same manner as *B. termo.* With this form alternates a zooglea form, where great numbers of immobile organisms are aggregated together within a delicate jelly-like mass. This organism is found in all putrefying animal matter, and is one of those associated with putrescent odours.

BACTERIUM LACTIS.—In milk, produces the characteristic odour and taste of sour milk, but does not cause coagulation, as many micro-organisms do.

Microscopically.—It consists of small dumb-bells, 2 μ. × 1 μ., which have very active movements. Here and there they are found aggregated into clusters, which seem to represent the zooglea stage of this organism.

BACTERIUM XANTHINUM.—Grows in boiled milk, producing a slight yellow colour. A small bacterium, apparently identical morphogically with *B. termo.*



BACILLI.

Longer or shorter cylindrical cells, growing longitudinally, and dividing by transverse cleavage. Ultimately they tend to grow into long threads (*Leptothrix*), along the course of which highly refractile round spores are produced. The spores are set free, and under favourable conditions reproduce the bacillus form. Bacilli often form clusters, but very seldom are found in zooglea masses.

BACILLUS SUBTILIS. *On Potato, &c.*—Forms a very moist, transparent, jelly-like film, which rapidly spreads itself over the nutrient surface, and has a clear glistening appearance.

In Gelatine.—It causes liquefaction slowly from above downwards, giving rise to a granular white precipitate at the bottom of the liquefied portions, and growing downwards in the tracks of the inoculating needle as dense opaque spikes.

Microscopically.—It consists of rods, usually about 2 μ. thick by 6 or 8 μ. long. Growth takes place rapidly; and at a slightly elevated temperature (35° C.) it is possible to observe the process of longitudinal growth and transverse cleavage, which accompanies the multiplication of these organisms, fully carried out within thirty or forty minutes (Flügge). In liquid media the organism moves actively by two flagella—one at each end. The spores are of an oval shape, and thicker than the rods.

BACILLUS ANTHRACIS. *On Potato* (see Fig. 15, p. 58).—It forms a cream-coloured layer of a succulent consistence, which grows rapidly at slightly elevated temperatures.

In Gelatine (see Fig. 37).—Causes liquefaction of the jelly, and the characteristic reactions described on p. 84.

In Liquid Media.—It forms a fine filmy cloud in the lower strata of the liquid, and after a time is deposited as a fine powder on the floor of the beaker in which it is cultivated.

Microscopically.—It is indistinguishable from *B. subtilis* in its rod-like stage. In liquid media the rods are described to be flagellate and mobile, and similar to *B. subtilis* in all respects. In its spore formation there is, however, a slight difference. The spores of *B. anthracis* are spherical, and are not thicker than the lengthened rods, in the continuity of which they appear; and in this stage the organisms can be easily differentiated.

BACILLUS TUBERCULOSIS. *On Serum* (see Fig. 55).—For description see p. 104. It refuses to grow well in other nutrient media.

Microscopically.—Consists of small thin nodules, often curved, and frequently possessing a bearded appearance, due to spore formation. The bacilli measure about 4 μ in length by 0·5 μ. in breadth, and the spores are not broader than the rods. For special methods of staining it, see § 27, p 39.

BACILLUS LEPRÆ. *Microscopically.*—Almost identical with the tubercle bacillus, but is found in different relations to the cell elements of its host.

BACILLUS MALARIÆ (Klebs).—A small organism found free in the Pontine Marshes and in the swamps of America, 2 to 7 μ. in length by 1 μ. in breadth, growing into long fibrils, and multiplying by transverse cleavage. By some it is supposed to be the cause of malaria.

BACILLUS OF BLUE MILK. *In Milk.*—A dark purple colour is produced by the rapid multiplication of the organism. The milk does not curdle, and retains its ordinary consistence.

In Gelatine.—A small rounded mass occupies the surface of the jelly at the seat of inoculation, and from this a delicate opaque spike passes downwards in the track of the inoculating wire, and the whole of the jelly becomes tinged with a peculiar green tint, which is modified according as it is viewed with direct or oblique illumination.

Microscopically.—Short rods 2·5 μ. long which are often connected end to end. *In fluid media* they perform rapid movements similar to those of *B. termo.*

FLUORESCING BACILLUS.—See description of Fig. 36, p. 81.

VIOLET BACILLUS.—See description of Fig. 35, p. 81.

BACILLUS OF MOUSE SEPTICÆMIA.—*In gelatine*, it grows rapidly, giving the appearance of a very attenuated cloud, most distinctly visible by oblique illumination. The appearance is due to the bacilli, each lengthening into a spiral fibril in the jelly mass.

Microscopically.—They are extremely small rods, only 1 μ. in length and 0·1 μ. in breadth, so that they are only to be seen with very high powers of the microscope. They occupy the *liquor sanguinis* and the interior of the white blood corpuscles of the blood of the house-mouse, but they are unable to develop themselves in that of the field-mouse.

MICROCOCCI.

MICROCOCCUS PRODIGIOSUS. *On Potato, Bread, &c.*—Forms a thick creamy film of an intensely bright crimson colour, which grows with great rapidity, and covers the whole of the nutrient surface (Fig. 19, p. 61).

In Gelatine.—It causes liquefaction rapidly, and gives its characteristic hue to the liquid portions.

In Milk, &c.—It produces a deposit with the typical crimson colour.

Microscopically.—Small round cocci, ½-1 μ. in diameter, which are not themselves pigmented, but which excrete the granular particles of pigment, which give the growth so distinctive an appearance.

MICROCOCCUS OF OSTEO-MYELITIS. *In Nutrient Media.*—See description, p. 86, and Figs. 29 and 38.

Microscopically.—A small spherical micrococcus found in large numbers in the pus from the abscesses of bone, which occur in cases of acute *osteo-myelitis*. They tend to form small circular clusters which float free in the *liquor puris*.

PYOGENETIC MICROCOCCI.

1. STAPHYLOCOCCUS PYOGENES ALBUS. *In Gelatine.*—Causes rapid liquefaction, forming a funnel-shaped depression in the line of the inoculating needle; at the bottom of this is seen a dense granular precipitate of a grey colour. The whole of the jelly soon becomes liquid.

On Sterile Serum.—It forms a pearly white succulent growth on the surface of the serum following the line in which the seed material was implanted.

Microscopically.—It consists of large irregular clusters of cocci (*Staphylococcus*) lying loose in the pus, or in some cases applied to and surrounding individual pus cells as a tunic. Found in contents of newly opened acute abscesses.

2. STAPHYLOCOCCUS PYOGENES AUREUS. *In Gelatine.*—Causes liquefaction rapidly, and a dense granular precipitate of an orange colour at the bottom of the liquid.

On Sterile Serum.—It forms a creamy growth of a bright orange colour, covering the surface of the serum at the point of inoculation.

Microscopically.—Irregular clusters of cocci, indistinguishable from the preceding species, except when cultivated artificially. It occurs in acute abscesses, whitlows, and boils.

3. STREPTOCOCCUS PYOGENES. *In Gelatine.*—Causes a funnel-shaped liquefied depression in the jelly, and forms a light yellow precipitate. The whole of the nutrient jelly in time becomes liquid.

On Serum.—It forms a delicate light yellow film, with a dry powdery consistence, growing in irregular serpentine patches on the surface of the serum.

Microscopically.—Long chains of cocci (*Streptococcus*) twisted and contorted in various directions, also in shorter chains, rarely isolated. It is found in *pyæmic* abscesses, and in the tissues in cases of moist gangrene.

LIST OF SOME ANTISEPTIC SUBSTANCES,

With the Minimum Degree of Concentration in Watery Solutions in which they are reliable (adapted from Miquel).

Oxygenated water (solution of peroxide of hydrogen),	1 to 2000
Perchloride of mercury,	1 „ 1425
Nitrate of silver,	1 „ 1250
Iodine,	1 „ 400
Perchloride of gold,	1 „ 400
Perchloride of platinum,	1 „ 333
Hydrocyanic acid,	1 „ 250
Bromine,	1 „ 166
Sulphate of copper,	1 „ 110
Chloroform,	1 „ 66
Chloride of zinc,	1 „ 52
Thymic acid,	1 „ 50
Carbolic acid,	1 „ 31
Permanganate of potash,	1 „ 28
Alum,	1 „ 22
Boric acid,	1 „ 13
Salicylate of soda,	1 „ 10

APPENDIX B.

LITERATURE.

In the following Bibliographical List, a certain classification has been attempted, but the authors of many of the larger works, and even papers, go over such an enormous mass of fact, detail, and experiment, that it is almost impossible in the space at command to mention each work under all the headings of the subject-matter of which it treats. Each work is therefore usually mentioned but once, and then under the name of the most important subject that is discussed in it. However incomplete the list may be, great care has been taken to make it accurate, and in nearly every instance the reference has been verified. The authors will be glad to receive additional references.

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